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Review-Resealed Erythrocyte as a Drug Carrier

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ABSTRACT

Resealed erythrocytes have been evaluated in thousands of drug administration in humans proving safety and efficacy of the treatments. Carrier erythrocytes, resealed erythrocytes loaded by a drug or other therapeutic agents, have been exploited extensively in recent years for both temporally and spatially controlled delivery of a wide variety of drugs and other bioactive agents owing to their remarkable degree of biocompatibility, biodegradability and a series of other potential advantages. 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. In this review article, 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.

Keywords: Resealed Erythrocytes, Morphology of erythrosites, Characterization of Resealed Erythrocytes, Storage of Erythrocytes

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INTRODUCTION

Erythrocytes, the most abundant cells in the human body, have potential carrier capabilities for the delivery of drugs. Erythrocytes are biocompatible, biodegradable, possess very long circulation half lives and can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods. Application of erythrocytes as promising slow drug release or site-targeted delivery systems for a variety of bioactive agents from different fields of therapy has gained a remarkable degree of interest in recent years. Biopharmaceuticals are among the most widely exploited candidates for being delivered to the host body using these cellular carriers. In this review, the potential applications of erythrocytes in drug delivery have been highlighted. 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.[1]

Erythrocytes

Red blood cells (also referred to as erythrocytes) are the most common type of blood cells and the vertebrate organism's principal means of delivering oxygen (O2) to the body tissues via the blood flow through the circulatory system. They take up oxygen in the lungs or gills and release it while squeezing through the body's capillaries. These cells' cytoplasm is rich in hemoglobin, an iron-containing bimolecule that can bind oxygen and is responsible for the blood's red color. In humans, mature red blood cells are flexible biconcave disks that lack a cell nucleus and most organelles.

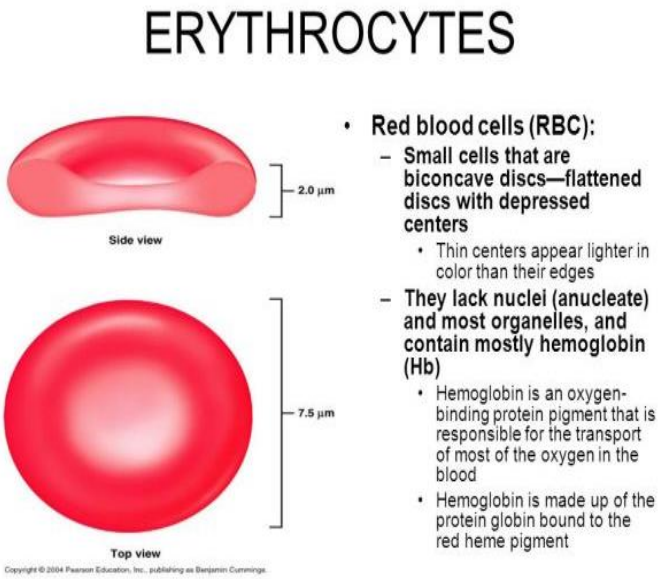

Figure 1: Morphology and physiology of erythrocytes

2.4 million new erythrocytes are produced per second. [2] Resealed Erythrocytes: 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 {#}[3]

Morphology and physiology of erythrocytes

Advantages of Resealed Erythrocytes

1. No chance of triggered immune response.
2. Biodegradability with harmful products or no generation of toxic products.
3. The considerable uniform size and shape of the carrier.
4. Relatively inert intracellular environment.
5. Prevention of degradation of the loaded drug from inactivation by endogenous chemicals.
6. The wide variety of chemicals and enzymes can be entrapped.
7. The modification of pharmacokinetic and pharmacodynamics parameters of drug can be done.
8. Attainment of steady-state plasma concentration which decreases fluctuations in concentration of drug.
9. Protection of the organism against toxic effects of drugs (e.g., antineoplastics)
10. Ease of circulation and ability to target RES organ
11. Prolong systemic activity of the drug while residing for a longer time in the body.
12. A remarkable degree of biocompatibility, particularly when the autologous cells are used for drug loading.
13. Complete biodegradability and the lack of toxic product(s) resulting from the carrier biodegradation.
14. Avoidance of any undesired immune responses against the encapsulated drug.
15. Considerable protection of the organism against the toxic effects of the encapsulated drug, e.g. antineoplasms.(4)

Disadvantages of Resealed Erythrocytes

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.

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 long.[7]

- Blood is collected into heparin zed tubes by venipunture.
- Blood is withdrawn from cardiac puncture (in small animal) and through veins (in large animals) in a syring containing a drop of anti-coagulant.
- The whole blood is centrifuged at 2500rpm for 5 min. at 4 ±10C in a refrigerated centrifuge.
- The serum and buffy coats are carefully removed and packed cells washed three times with phosphate buffer saline pH=7.4).
- The washed erythrocytes are diluted with phosphate buffer solution and stored at 4oC until used.
- Various types of mammalian erythrocytes have been used for drug delivery, including erthrocytes of mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits. The encapsulation efficiency of the erythrocytes isolated from fresh blood is more than that of the aged blood. 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 erythrocytes are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid–citrate–dextrose buffer at 40C for as long as 48 h before use. Entrapment of dextran (molecular weight10–250 kDa) and loading of drugs in erythrocytes was reported separately.[8-9]

METHODS OF DRUG LOADING:-

1] Hypotonic Heamolysis

Erythrocytes have an outstanding capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume

leads to an first change in the shape from biconcave to spherical. This change is attributable to the absence of unnecessary membrane; hence, the surface area of the cell is preset. The cells assume a spherical shape to accommodate additional volume while observance the surface area constant. The volume gain is 25–50%. This method is based on the ability of erythrocytes to under reversible inflammation in a hypotonic solution. The cells can keep up their integrity up to a tonicity of 150 mos m/kg, above which the membrane ruptures, releasing the cellular contents.

On this position (just before Cell lysis), some passing pores of 200–500 Å are generated on the membrane. After cell lysis, cellular contents are exhausted the remains is called an erythrocyte ghost. Upon incubation; the cells resume their exclusive biconcave shape and recover unique impermeability.

2] Use of red cell loader

New method was developed for entrapment of nondiffusible drugs into erythrocytes. They developed a portion of equipment called a "red cell loader. With as slight as 50 ml of a blood sample, dissimilar 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 in order hypotonic dilutions of washed erythrocytes followed by concentration with a hemofilter and an isotonic resealing of the cells. There was 35–50% cell recovery with 30% drug loading. The processed erythrocytes had usual survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages.

3] Hypotonic dilution

In this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug. The solution tonicity is then restored by adding a hypertonic buffer. The resultant mixture is then centrifuged, the supernatant is discarded, and the pellet is washed with isotonic buffer solution.[10]

4] Hypotonic pre-swelling

This method was developed by Rechsteiner in 1975 and was developed by Jenner et al. for drug loading. The technique is based upon initial controlled inflammation in a hypotonic buffered solution. This mixture is centrifuged at low *g*values. The supernatant is not needed and the cell fraction is brought to the lysis point by adding 100–120 Ltrs portions of an aqueous solution of the drug to be encapsulated. The mixture is centrifuged among the drug-addition steps. The lysis point is detected by the disappearance of a distinct limit between the cell portion and the supernatant upon centrifugation. The tonicity of a cell mixture is restored at the lysis point by adding a calculated quantity of hypertonic buffer. They cell have a circulation half-life comparable to that of

normal cells. This method is simpler and faster than other methods, causing lowest amount injure to cells. Drugs encapsulated in erythrocytes using this technique include propranolol, asparginase, cyclopohphamide, 1-antitrypsin, methotrexate, insulin, metronidazole, levothyroxine, enalaprilat, and isoniazid.[11-13]

5] Hypotonic dialysis

This method was first reported by Klibansky in 1959 and was used in 1977 by Deloach, Ihler and Dale for loading enzymes and lipids. In the process, an isotonic, buffered suspension of erythrocytes with a hematocrit value of 70–80 is prepared and placed in a conventional dialysis tube immersed in 10–20 volumes of a hypotonic buffer. The medium is agitated slowly for 2 h. The tonicity of the dialysis tube is restored by directly adding a calculated amount of a hypertonic buffer to the surrounding medium or by replacing the surrounding medium by isotonic buffer. The drug to be loaded can be added by either dissolving the drug in isotonic cell suspending buffer inside a dialysis bag at the beginning of the experiment or by adding the drug to a dialysis bag after the stirring is complete.[19]

6] Isotonic osmoticlysis

This method was reported by Schrier et al in 1975.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 sealed at 37oC.[14-18]

7] Chemical perturbation of the membrane

This process is based on the raise in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. Amphotericin B. such as permeability of erythrocytic membrane increases upon exposure to polyene antibiotic In 1980, this technique was used successfully to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes. However these process induce irreversible critical changes in the cell membrane and hence are not very popular.

8] Electro-insertion or electro encapsulation

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. This method is also called as electroporation. In this method erythrocyte membrane is open by a dielectric breakdown; subsequently the pore of erythrocyte can be resealed by incubation at 370C in an isotonic medium. The various chemical encapsulated into the erythrocytes are primaquin and related 8- amino quinolone, vinblastin chloropromazine and related phenothiazine, propanolol, tetracaine and vitamin A.[39-41]

9] Entrapment by endocytosis

This process was reported by Schrier et al. in 1975. Endocytosis involves the addition of one volume of washed filled 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 technique are resealed by using 154 mm of NaCl and incubation at 37oC for 2 min. The setup of material occurs by endocytosis. The vesicle membrane separates endocytosed substance from cytoplasm thus defensive it from the erythrocytes and vice-versa. The various candidates entrapped by this method include primaquine and related 8–amino– quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A.

10] Loading by electric cell fusion

This process 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 purpose of an electric pulse, which causes the release of an entrapped molecule. An example of this process is loading a cell-specific monoclonal antibody into an erythrocyte ghost. An antibody against a specific shell protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells. [21]

11] Loading by lipid fusion

The lipid vesicles containing a drug can be directly fuse to human erythrocytes, which lead to an exchange with a lipid 161 entrapped drug. The methods are useful for entrapping inositol monophasphte to improve the oxygen carrying capacity of cells and entrapment efficiency of this method is very low (~1%).[20]

Characterization of Resealed Erythrocytes:-

These characterizations are important to ensure their in-vivo performance and therapeutic benefits.

I. Drug Content Determination

Method: 0.5ml packed loaded erythrocytes are deproteinized with acetonitrile (2 ml) and then centrifuged at 2500 rpm for 10 minutes. Now the clear supernatant liquid is analyzed for drug content.

II. *In-vitro* drug release and Hb content: Both these properties are monitored periodically from drug-loaded cells

Method: The cell suspension (5% Haematocrit in Phosphate buffer saline) is stored at 4oC in amber colored glass containers. Periodically the clear supernatant are withdrawn using a hypodermic syringe equipped with 0.45µ filter, deproteinized with methanol and then estimated for drug content. The supernatant of each sample after centrifugation is collected and assayed. Hence, % Hb (Haemoglobin) release is calculated.

% Hb release= A540 of sample - A540 of background A540=Absorbance at 540 nm, A540 of 100% Hb Mean Corpuscular Hb = Hb (g/100 ml) × 10 Erythrocyte count (millions/cu mm)

III. Percent cell recovery

Percent cell recovery determine by counting the number of whole cells per cubic mm of packed erythrocytes before and after loading the drug.

IV. Morphology

Following types of microscopy are used for the morphological study of normal and drug loaded erythrocytes:

i. Phase contrast microscopy

ii. Electron microscopy

a. Scanning electron microscopy

b. Transmission electron microscopy

V. Osmotic fragility

This method is based on resistance of cells to haemolysis in decreasing concentration of hypotonic saline.

It is a reliable parameter for:

□□*In-vitro* evaluation of carrier erythrocytes with respect to shelf life

□□*In-vivo* survival of erythrocytes

□□Study of effect of the encapsulated substances

□□For stimulating and mimicking the bio-environmental conditions that are encountered on in-vivo administration.

Method: Normal and drug - loaded erythrocytes are incubated separately in stepwise decreasing % of NaCl solution (0.9%) at 37oC±2oC for 10 minutes , followed by centrifugation at 2000 rpm for 10 minutes. Then the supernatant liquid is examined for drug and haemoglobin content.

VI. Osmotic shock

This is used to describe a sudden exposure of drug loaded erythrocytes to an environment, which is

far from isotonic so as to calculate the capability of resealed erythrocytes to withstand the stress and keep up their integrity as well as appearance.

Method: Erythrocyte suspension (10% haematocrit,1 ml) was diluted with distilled water(5 ml) and centrifuged at 300 rpm for 15 minutes. Supernatant was estimated for % Hb release spectrophotometrically.

VII. Turbulence shock

This parameter indicates the effects of shear and pressure, by which resealed erythrocyte formulations are injected, on the integrity of the loaded cells. Drug loaded erythrocytes appear to be less resistant to turbulence because resealing of erythrocytes make them sensitive towards turbulence/ Mechanical agitation and hence estimation of turbulence shock study provides their expected performance in-vivo.

Method: Loaded erythrocytes(10% haematocrit,5 ml) are passed through 23-gauge hypodermic needle at a flow rate of 10 ml/minute(which is comparable to the flow rate of blood).After every pass, aliquot of suspension is withdrawn and then centrifuge at 2000 rpm for 10-15 minutes. Now the Hb content is estimated spectrophotometrically.

VIII. Determination of entrapped Magnetite

Resealed erythrocytes are entrapped with magnetite to make them Magnoresponsive.

Method: Magnetite bearing erythrocytes and Hydrochloric acid are heated at 60ºC for 2 hour. Now 20% w/v trichloroacetic acid is added. Centrifugation is done and supernatant is examined for Magnetite concentration using atomic absorption spectroscopy.

IX. Erythrocyte Sedimentation Rate (ESR)

ESR is the estimation of suspension stability of RBC in plasma and is related to:

- Number and size of red cells.
- The relative concentration of plasma proteins (especially fibrogen ,alpha and beta globulins)

This test is performed by determining the ESR of blood cells in a standard tube of ESR apparatus. Higher rate of ESR is indication of active but obscure disease processes. The normal blood ESR is found to be 0 to 15 mm/hour.

X. The Zeta Sedimentation Ratio

It is based on a measure of the closeness with which RBC's will approach each other after standardized cycles of dispersion and compaction.

XI. Miscellaneous

Lipid composition, Membrane fluidity, rheological properties, density gradient separation, energy metabolism, Biological characterization(sterility test using aerobic and anaerobic cultures,

Pyrogenicity using rabbit fever response or LAL test, animal toxicity study)

Storage of Erythrocytes:

The storage of resealed erythrocytes places a major challenge in their practical utility as drug delivery system and is important pre-requisite for the erythrocytes as a drug carrier. The Encapsulated preparation should be stored in such a way that there should be no loss of integrity. There are following methods for the storage of loaded erythrocytes: I. Suspending in Hank's balanced salt solution at 4oC for two weeks.

II. After encapsulation suspending the cells in oxygenated Hank's balanced salt solution containing 1% soft bloom gelatin. The cells can be recovered by liquefying the gel by placing the tubes in water bath at 37oC and centrifugation under clinical conditions.

III. or Cryopreservation of erythrocytes at liquid nitrogen temperature.

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.[36-37]

APPLICATIONS:-

a. Slow Drug Release

Erythrocytes have been used as storehouse for the sustained delivery of various drugs like anticancer, antiparasitic, veterinary, anti amoebic,vitamins, steroids, antibiotics and cardiovascular drugs.[33]

b. Drug Targeting

Ideally, drug delivery should be site-specific and target-oriented to show maximal therapeutic index with least undesirable effects. Resealed erythrocytes can act as drug carriers and targeting tools also. Surface-tailored erythrocytes are used to target organs of mononuclear phagocytic system/ reticuloendothelial system because the changes in the membrane are renowned by macrophages. [34]

c. Targeting RES organs

Damaged erythrocytes are quickly blank from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes by developed their membranes, can therefore be used to target the liver and spleen. The various approaches to modify the surface characteristics of erythrocytes

include:

- Surface modification with antibodies
- Surface modification with gluteraldehyde
- Surface modification with carbohydrates such as sialic acid
- Surface modification with sulphydryl
- Surface chemical cross-linking e.g. Delivery of 125i-labeled carbonic anhydrase loaded in erythrocytes cross-linked with bis (sulfosuccinimidyl) suberate and 3, 3- dithio (sulfosuccinmidyl propionate).[35]

d. Targeting the liver, Enzyme deficiency/ replacement 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 effectively overcome by administer the enzymes as resealed erythrocytes. The enzymes used include -glucosidase, -glucoronidase, -galactosidase. The disease caused by an accumulation of glucocerebrosidase in the liver and spleen can be treated by glucocerebrosidase- loaded erythrocytes.[24]

e. 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 or cis-aconitic acid as a spacer. The resealed erythrocytes loaded with carboplatin show localization in liver.[22-23]

f. Treatment of parasitic diseases

The ability of resealed erythrocytes to selectively mount up within reticule endothelial system organs make them useful tool during the delivery of antiparasitic agents. Parasitic diseases that engage harboring parasites in the RES organs can be successfully restricted by this method. Results were positive in studies concerning animal models for erythrocytes loaded with antimalarial, antileishmanial, and antiamoebic drugs.[25]

g. Removal of reticule endothelial system iron overload

Desferrioxamine (iron-chelating agent) loaded erythrocytes have been used to be concerned for excess iron accumulated because of various transfusions to thalassemic patients. Targeting this drug to the RES is very useful because the aged erythrocytes are spoiled in RES organs, which

results in an accumulation of iron in these organs.

h. Removal of Toxic Agents

Cannon et al. reported inhibition of cyanide intoxication with murine carrier erythrocytes containing bovine rhodanase and sodium thiosulphate. Antagonization of organophosphorus intoxication by resealed erythrocytes containing a recombinant phosphodiestrase also has been reported.[26-27]

i. Targeting organs other than those of RES

The various approaches include:

- Entrapment of paramagnetic particles along with the drug
- Entrapment of photosensitive material
- The use of ultrasound waves
- Antibody attachment to erythrocyte membrane to get specificity of action.

j. Delivered 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.[35]

k. Enzyme 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 injection of enzymes into the body have been cited. One method to conquer these problems is the use of enzyme-loaded erythrocytes. These cells then release enzymes into circulation upon hemolysis and act as a "circulating bioreactors" in which substrates enter into the cell, interact with enzymes, and generate products or accrue 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 –glucosere brosidase for the treatment of Gaucher's disease. The disease is characterized by inborn deficiency of lysosomal- glucosere brosidase in cells of RES thereby leading to accumulation of glucosere brosides in macrophages of the RES. This leads to an growth of aminolevulinic acid in tissues, blood, and urine. This state leads to delicate porphyria and CNS related difficulty.[28]

l. **Improvement in oxygen delivery to tissues**

Haemoglobin is the protein responsible for the oxygen-carrying capacity of erythrocytes. Under normal conditions, 95% of haemoglobin is saturated with oxygen in the lungs, whereas under physiological conditions, in peripheral blood stream, only 25% of oxygenated haemoglobin becomes deoxygenated. Thus, the major fraction of oxygen bound to haemoglobin is recirculated with venous blood to the lungs.

B. In-vitro Applications

Most important in-vitro application is that of Microinjection of macromolecules. DNA, RNA, and proteins are exploited for different cell biological applications. Hence, various methods are used to entrap these macromolecules into cultured cells (e.g., microinjection).

C. In microinjection, erythrocytes are used in micro- syringes for injection in the host cells. The microinjection method involves culturing host eukaryotic cells *in vitro*. The cells are coated with fusogenic agent and then suspended with erythrocytes full with the compound of interest in an isotonic medium. Sendai virus its glycoprotein's or polyethylene glycol has been used as fusogenic agents. The fusogen causes fusion of co-suspended erythrocytes and eukaryotic cells.

Novel Approaches (Recent Developments)

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. [29-30]

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.[31-32]

CONCLUSION

During the past decade, numerous applications have been proposed for the use of resealed erythrocytes as carrier for drugs, enzyme replacement therapy etc. 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 and much remains to be explored regarding the potential of resealed erythrocytes. For the present, it is concluded that erythrocyte carriers are "golden eggs in novel drug delivery systems" considering their tremendous

potential. Most of the studies in this area are in the in vitro phase and the ongoing projects worldwide remain to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

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