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Review Article

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Resealed Erythrocytes: A Novel Drug Delivery System

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ABSTRACT

Now days the research work in the drug development is mainly focusing on targeted drug delivery for better therapeutic effect. Carrier 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.

Key-words: Resealed erythrocytes, Erythrocytes, Drug Carriers, Characterization methods and Applications

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INTRODUCTION:-

To achieve a required therapeutic concentration the drug has to be administered in large quantities, the major part of which is just wasted in normal tissues. Ideally, a "perfect" drug should exert its pharmacological activity only at the target site, using the lowest concentration possible and without negative effects on non-target compartments. The delivery systems currently available enlist carriers that are either simple, soluble macromolecules (such as monoclonal antibodies, soluble synthetic polymers, polysaccharides and particulate biodegradable polymers) or more complex multicomponent structures (microcapsules, microparticles, cells, cell ghosts, lipoproteins, liposomes, erythrocytes)[1]. Erythrocytes, also known as red blood cells, have been extensively studied for their potential carrier capabilities for the delivery of drugs and drug-loaded microspheres. 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. 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 areticulo endothelial system.

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[2].

MORPHOLOGY AND PHYSIOLOGY OF ERYTHROCYTES:-[2]

- Erythrocytes are the most abundant cells in the human body. These are biconcave discs with an average diameter of 7.8μm, a thickness of 2.5 m in periphery.
- The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries, which may be only 3μm wide. Mature erythrocytes are quite simple in structure.
- They lack a nucleus and other organelles. Their plasma membrane encloses hemoglobin, a heme-containing protein that is responsible for O_2–CO_2 binding inside the erythrocytes.
- Erythrocytes are highly specialized O_2 carrier system in the body. Because a nucleus is absent, all the intracellular space is available for O_2 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.

Properties of resealed erythrocyte of novel drug delivery carriers:-[4]

- The drug should be released at target site in a controlled manner.
- It should be appropriate size, shape and should permit the passage through capillaries and minimum leakage of drug should take place.

- It should be biocompatible and should have minimum toxic effect.
- It should possess the ability to carry a broad spectrum of drug.
- It should possess specific physicochemical properties by which desired target size could be recognized.
- The degradation product of the carriers system release of the drug at the selected site should be biocompatible. It should be physicochemically compatible with drug.
- The carrier system should have an appreciable stability during storage.

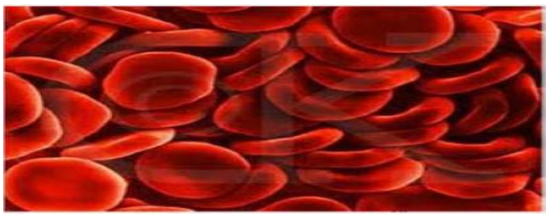

Figure No. 1: Erythrocytes[3].

ADVANTAGES OF RESEALED ERYTHROCYTES AS DRUG CARRIERS:-[5]

1. Their biocompatibility, particularly when autologous cells are used, hence no possibility of triggered immune response.
2. Their biodegradability with no generation of toxic products.
3. The considerably 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 modification of pharmacokinetic and pharmacodynamic parameters of drug.
7. Attainment of steady-state plasma concentration decreases fluctuations in concentration.
8. Protection of the organism against toxic effects of drugs (e.g. antineoplastics).
9. They are ability to circulate throughout the body the availability of the techniques and facilities for separation, handling, transfusion, and working with erythrocytes.
10. Improvement in oxygen delivery to tissues.
11. The prevention of any undesired immune response against the loaded drug.
12. Their ability to target the organs of the RES.
13. The possibility of ideal zero-order drug-release kinetics.
14. The lack of occurrence of undesired immune response against encapsulated drug.
15. The large quantity of drug that can be encapsulated within a small volume of cells ensures dose sufficiency.
16. 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.
17. Easy control during life span ranging from minutes to months.
18. A decrease in side effects of drugs.
19. A considerable increase in drug dosing interval with drug residing in therapeutic window region for longer time periods.

DISADVANTAGES OF RESEALED ERYTHROCYTES AS DRUG CARRIERS:-[2]

1. 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 life-span as long-circulating drug carriers in circulation and, in some cases, may pose toxicological problems.

2. The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
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. 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 temperatures, the addition of nucleosides or chelators, lyophilization with glycerol or gel immobilization have all been exploited to overcome this problem.
6. Possible contamination due to the origin of the blood, the equipment used and the loading environment.

ERYTHROCYTES:-[6]

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.

2. For 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.

Source 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.

Isolation of erythrocytes:-[3]

- Blood is collected into heparinized tubes by veinpuncture.
- Blood is withdrawn from cardiac/sphlenic puncture (in small animal) and through veins (in large animals)in a syringe containing a drop of anti-coagulant.
- The whole blood is centrifuged at 2500 rpm for 5 min. at 4 ±1^{0}C 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 PBS and stored at 4^{0}C for as long as 48 hr before use.

S.No	Species	Washing buffer	Centrifugal force(g)
1	Rabbit	10mmol KH2PO4/NaHPO4	50021000
2	Dog	15mmol KH2PO4/NaHPO4	50021000
3	Human	154mmol NaCl	<500
4	Mouse	10mmol KH2PO4/NaHPO4	1002500
5	Cow	10215mmol KH2PO4/NaHPO4	1000
6	Horse	2mmol MgCl2,10mmol glucose	1000
7	Sheep	10mmol KH2PO4/NaHPO4	50021000
8	Pig	10mmol KH2PO4/NaHPO4	50021000

Table No. 1: Various condition and centrifugal force used for isolation of erythrocytes[7].

Requirement for encapsulation:-[3]

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 Hydrochloride 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 β- galactosidase.

METHODS OF DRUG LOADING IN RESEALED ERYTHROCYTES:-[3,8]

Several methods can be used to load drugs or other bioactive compounds in erythrocytes, including physical (e.g., electrical pulse method) osmosis-based systems, and chemical methods (e.g., chemical perturbation of the erythrocytes membrane). Irrespective of the method used, the optimal characteristics for the successful entrapment of the compound requires the drug to have a considerable degree of water solubility, resistance against degradation within erythrocytes, lack of physical or chemical interaction with erythrocyte membrane, and well defined pharmacokinetic and pharmacodynamics properties.

Types:-

[A] Osmosis based methods
1] Hypotonic Hemolysis
2] Hypotonic Dilution
3] Hypotonic Dialysis
4] Hypotonic Pre-Swelling
B] Chemical perturbation of the membrane
C] Electro-insertion or electro encapsulation
D] Entrapment by endocytosis

[A] OSMOSIS BASED METHODS:-

1] Hypotonic Hemolysis:- This method is based on the ability of erythrocytes to undergo reversible swelling in a hypotonic solution as shown in figure. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under the cells can maintain their integrity up to a tonicity of 150mosm/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 A^0 are generated on the membrane Stress.

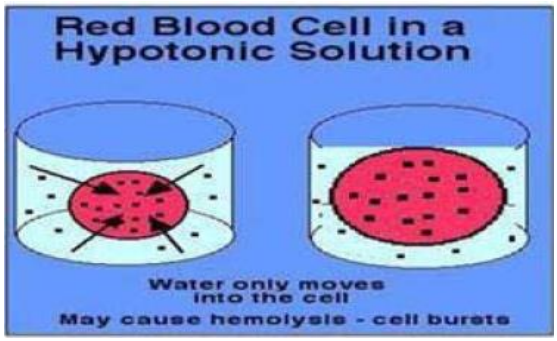

Figure No. 2: Hypotonic Hemolysis Method.

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

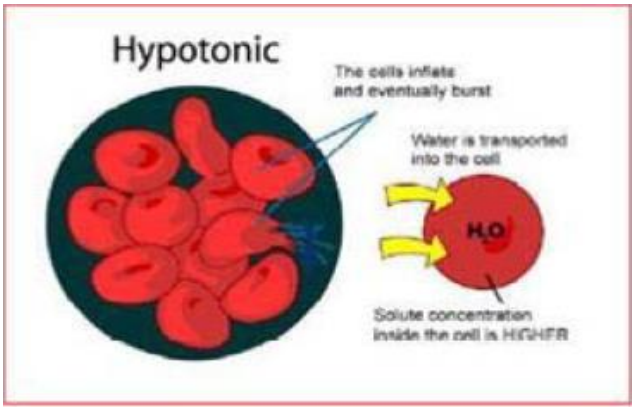

Figure No. 3: Hypotonic Dilution Method.

3] Hypotonic Dialysis:-Several methods are based on the principle that semi-permeable dialysis membrane maximizes the intracellular:extracellular volume ratio for macromolecules during lysis and resealing. In this process a desired hemocrit is achieves by mixing erythrocyte suspension and drug solution. This mixture is placed into dialysis tubing and then both ends of tube are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. The tube is placed in the bottle containing 100ml of swelling solution. The bottle is placed at 4^0C for the desire lysis time. The contents of the dialysis tubing are mixed intermittently by shaking the tube using the strings. The dialysis tube is then placed in 100 ml of resealing solution. The loaded erythrocytes thus obtained are then washed with cold phosphate buffer at 4°C. In this method a good entrapped efficiency is obtained.

4] Hypotonic Pre–Swelling:-As shown in Figure no. 4 the technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low g values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 L portions of an aqueous solution of the drug to be encapsulated.

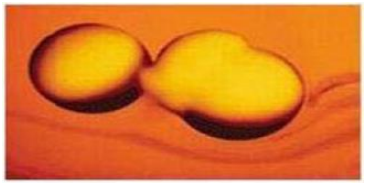

Figure No. 4: Hypotonic Pre - Swelling Method.

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 erythrocyte membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse erythrocytes these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

C] ELECTRO-INSERTION OR ELECTRO ENCAPSULATION:-

The method is based on the observation that electrical shock brings about irreversible changes in the erythrocyte membrane is opened by a dielectric breakdown. Subsequently, the pores can be resealed by incubation at 37°C in an isotonic medium. The procedure involves suspending erythrocytes in an isotonic buffer in an electrical discharge chamber. A capacitor in an external circuit is charged to a definite voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential. The optimum intensity

of an electric field is between 1–10kW/cm and optimal discharge time is between 20–160 μs. An inverse relationship exists between the electric-field intensity and the discharge time. The compound to be entrapped is added to the medium in which the cells are suspended from the commencement of the experiment.

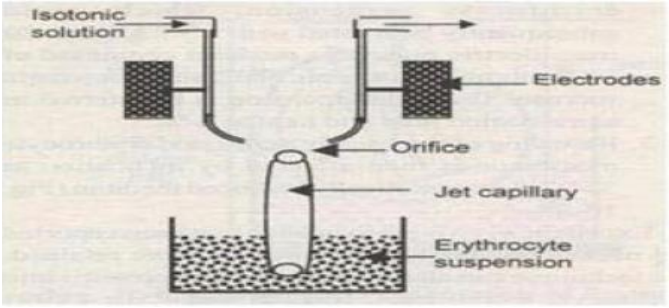

Figure No. 5: Electro-Insertion or Electro Encapsulation Method.

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

D] ENTRAPMENT BY ENDOCYTOSIS:-

This method was reported by Schrier in 1975. Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mMATP, 2.5mM $MgCl_2$ and 1mM $CaCl_2$ followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37°C for 2 min. The entrapment of material occurs by endocytosis. The vesicle membrane separates endocytosis material from cytoplasm thus protecting it from the erythrocytes and vice-versa.

DRUG RELEASE CHARACTERSTICS OF LOADED ERYTHROCYTES:-

There are mainly three ways for a drug to efflux out from erythrocyte carrier's i.e. phagocytosis, diffusion through the membrane of the cell and use of specific transport system. The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid bilayer it is greatest for molecule with high lipid solubility and gradually goes down with polarity or charged groups of the molecule. Prolongation of release could presumably be accomplished by entrapment of potent inhibitors of the appropriate transport protein along with the drug.

IN-VITRO CHARACTERISATION:-

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

A] Physical Characterization:-

1] Shape and Surface Morphology:-The morphology of erythrocytes decides their life span after administration. Light microscopy reveals no observable change in resealed cells but in few cases spherical erythrocytes (spherocytosis) are detected. Scanning electron microscopic studies have shown that a majority of the cells maintain their biconcave discoid shapes after the loading procedure, and few stomatocytes a form of spherocytosis with an invagination in one point are formed. In some cases, cells of smaller size (microcyte) are also observed.

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

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

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

1) The rate of drug release is considerably higher than that of hemoglobin. In other words, drug diffuses readily. Such a pattern is shown by lipophilic drugs, including methotrexate, phenytoin, dexamethasone, primaquin and vitamin B12.Cell lysis is not essential for the release of such drugs.

2) The rate of drug release is comparable to that of hemoglobin. This indicates that cell lysis is essential for drug release and drug cannot be released by mere diffusion. Polar drugs such as gentamicin, heparin, enalapril and enzymes such as asparginase peptides, including urogasterone and lysine- l-phenylalanine follow such pattern.

3) The rate of drug release lies between the above mentioned two extremes; for example, propranolol, isoniazid, metronidazole and recombinant human erythropoietin.

B] Cellular characterization:-

1] Osmotic Fragility:-The osmotic fragility of resealed erythrocytes is an indicator of the possible changes in cell membrane integrity and the resistance of these cells to osmotic pressure of the suspension medium. The test is carried out by suspending cells in media of varying sodium chloride concentration and determining the hemoglobin released. In most cases, osmotic fragility of resealed cells is higher than that of the normal cells because of increased intracellular osmotic pressure.

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

3] Percent cell recovery:-It can be determined by counting the number of intact cells per cubic mm of packed erythrocyte before and after loading the drug.

C] Biological characterization:-

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

IN-VIVO CHARACTERISATION:-

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

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 RBC's in liquid nitrogen.

APPLICATIONS OF RESEALED ERYTHROCYTES:-[9]

In-Vitro Applications:-

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.

In-Vivo 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 glucocerebrosidase 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 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.

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.

7) Treatment of hepatic tumors:- Antineoplastic drugs such as methotrexate(MTX), bleomycin, asparginase and adiramycin have been successfully delivered by erythrocytes. E.g. in a study, the MTX showed 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-Glcoside, β-glucouronidase, 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 attachment 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.

NOVEL APPROACHES:-

Erythrosomes:- These are specially engineered vesicular systems that are chemically cross-linked to human erythrocyte 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.

FUTURE PERSPECTIVES:-

- The concept of employing erythrocytes as drug or bioactive carrier still needs further optimization.
- A large amount of valuable work is needed so as to utilize the potentials of erythrocytes in passive as well as active targeting of drugs.
- The resealed erythrocytes can be utilized for in humans as carriers for drugs.
- Scientists have demonstrated that such engineered red blood cells are suitable for blood transfusion.

Future studies would concentrate on the following:-[2]

1. Manipulation of autologous properties of erythrocytes, improved understanding of the biology of the red cells and its membrane development of pulsatile and feedback control system, selective drug delivery to CNS and delivery peptide and protein drugs.
2. Technical improvement in the procedure for preparing resealed erythrocytes , routes of administration, stability, crosslinking of resealed erythrocytes, aseptic and sterile processing, optimization techniques , pilot- plant scale up studies and innovative ideas for the application of resealed erythrocytes. Either as carriers or as cellular bioreactors would pave the way for automation and commercialization of this novel drug delivery system.
3. With the availability of technology to clone human DNA prokaryotes and the potential to produce large quantity of human enzymes, the possibility of enzyme replacement therapy targeting and the use of RBC carrier reservoirs should become more of realties.
4. In future greatest interest seems to be related to the targeting of immune-modulators on the phagocytic system anticancer drugs.
5. Drug loaded magnetite bearing cells serve as a promising carrier for delivering the drug to specific site.

CONCLUSION:-

The various latent biomedical applications of RBCs based drug delivery systems opening new perspectives to the vision of using our cells for salutary purposes. Thus, the resealed erythrocyte is the promising carrier for various drugs, therapeutic proteins, a vaccine for both targeting and delivery. 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 custom 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 stay behind to step into preclinical and, then, clinical studies to prove the capabilities of this promising delivery system.

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