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Review Article

Resealed Erythrocytes: As a Carrier for Drug Targeting

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ABSTRACT

Now a day's most of the investigations are focused only on the development of various drug delivery systems for targeting of the desired tissue to achieving required therapeutic concentration with less amount of dose. Erythrocytes are biocompatible, biodegradable, possess long circulation half lives, and can be loaded with a variety of biologically active compounds using various chemical and physical methods. Erythrocytes have been the most interesting carrier and have found to possess great potential in drug targeting. Resealed erythrocytes are gaining more popularity because of their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation. Most of the resealed erythrocytes used as drug carriers are rapidly taken up from blood by macrophages of reticuloendothelial system (RES), which is present in liver, lung, and spleen of the body. The aim of the present review is to focus on the various features, drug loading technology and biomedical application of resealed erythrocytes.

Keyword: *Erythrocytes, Resealed Erythrocytes, Carrier Drug Targeting.*

INTRODUCTION:

Targeting of an active biomolecule 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[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.

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Hence, these carriers are called resealed erythrocytes. Various carriers has been used for the drug targeting among Which cellular carrier offer a greater potential advantages related to its biodegradability, non pathogenicity, non-immunogenicity, biocompatibility, self degradability along with high drug loading efficiency[2]. Among those Resealed erythrocytes are gaining more popularity as targeted drug carriers, due to their ability to circulate throughout the body, biocompatibility, zero order release kinetics, reproducibility and ease of preparation[3].

ERYTHROCYTES:-

Erythrocytes are natural products of the body, biodegradable in nature, isolation of these is easy and large amount of drug can be loaded in small volume of cells, non immunogenic in action and can be targeted to disease tissue or organ, prolong the systemic activity of the drug while residing for a longer time in the

body, protect the premature degradation, inactivation and excretion, of proteins and enzymes, act as a carrier for number of drugs, target the drugs within the reticuloendothelial system (RES) as well non RES organs/sites. They have the capacity to carry large amounts of drug; and can behave as a slow-release long acting system. Potential clinical indications for "RES targeting" include iron over-storage diseases, parasitic diseases, hepatic tumors, cancer and lysosomal storage diseases carriers.[4]

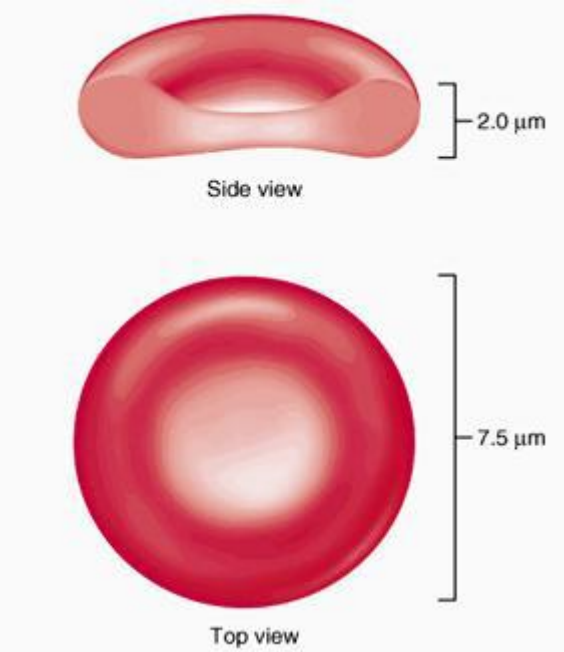

FIGURE 1: STRUCTURE OF ERYTHROCYTES

Composition of Erythrocytes:

Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements. Normal blood cells have extensile, elastic, biconcave and non nucleated configuration with a diameter ranging from 6-9 µ and the thickness is nearly 1-2 µ. Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water. Lipid content of erythrocytes includes cholesterol, lecithin and cephaelins.[5]

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 carriers8. 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 a reticuloendothelial system (RES).[6]

Various advantages of resealed erythrocytes: [22]

- They are the natural product of the body, which are biodegradable in nature.
- Isolation of erythrocytes is easy and larger amount of drug can be encapsulated in a small volume of cells.
- The entrapment of drug does not require the chemical modification of the substance to be entrapped. This is in contrast with other systems which involve covalent coupling of the drug and carrier which may effect the inherent biological activity of the parent drug.
- They are non-immunogenic in action and can be targeted to disease tissue/organ.
- They prolong the systemic activity of drug while residing for a longer time in the body.
- They protect the premature degradation, inactivation and excretion of proteins and enzymes and act as a carrier for number of drugs.
- They can target the drug within reticuloendothelial system (RES).
- They facilitate incorporation of proteins and nucleic acid in eukaryotic cells by cell infusion with RBC.

Disadvantages of resealed erythrocytes: [23-27]

- 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.
- The rapid leakage of certain encapsulated substances from the loaded erythrocytes.
- Several molecules may alter the physiology of the erythrocyte.
- 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.

- 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.
- 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.

Isolation of Erythrocytes: [7]

- Blood is collected into heparin zed tubes by venipunture.
- 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.
- The whole blood is centrifuged at 2500 rpm for 5 min. at 4±10oC.
- The serum and buffy coats are carefully removed and packed cells washed three times with phosphate buffer saline (pH=7.4). 4±10oC in a refrigerated centrifuge.
- The washed erythrocytes are diluted with PBS and stored at 4°C until used.

Drug loading can be done by immersing the cells in buffered hypotonic solution of drug which causes them to rupture and release haemoglobin and trap the medicament. On restoration of isotonicity and incubation at 37C, the cells reseal and are ready for use. Upon re-injection, the drug loaded erythrocytes serve as slow circulating depots. Damaged erythrocytes are removed by the liver and spleen. These organs can thus be specifically targeted by drug loaded erythrocytes and is used in the therapy such as enzyme replacement, treating liver tumours, eradication of parasites etc. [9]

Factors which considering resealed erythrocytes as carrier:[8]

- Appropriate size(s) and shape to permit the passage through the capillaries.
- It should have minimum side effects and biocompatible.
- It should have specific physicochemical properties by which a desired target site could be recognized.
- Drug should be released at the target site in a controlled manner.
- Low leaching/leakage of drug should take place before target site is reached.
- Physico-chemical compatibility with the drug.
- It should possess the ability to carry a wide variety of drugs with different properties.
- It should have sufficient space to carry and eventually to permit the delivery of clinically adequate amounts of drug.
- The carrier system should have an appreciable stability during storage.

METHODS OF DRUG LOADING IN ERYTHROCYTES:

Several methods can be used to load drugs or other bioactive compounds in erythrocytes, the following are types of drug loading:

Hypo-osmotic lysis method: [10,11]

Hypotonic lysis of cells in a solution containing the drug/enzyme to be entrapped followed by restoration of tonicity in reseal them. The ghost populations obtained are heterogeneous and they are three types. The type I ghosts which reseal immediately after haemolysis, type II ghosts which reseal after reversal of haemolysis by addition of alkali ions and type III ghosts which remain leaky under different experimental conditions. Erythrocytes have capability to reversible shape change with or without accompanying volume change. They don't have internal membrane and no capacity to synthesize additional plasma membranes, the surface area is inevitably fixed, so Increase in volume initially leads to conversion of normal erythrocyte to spherocytes. These swollen erythrocytes 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 rupture, permitting escape of the cellular component Erythrocyte are resealed on addition of sufficient1. 54 M KCl, which restores isotonicity. in experiments, where preservation of energy metabolism within the cells is desirable, 4mM magnesium salts, 10 Mm glucose and 2mM adenosine are included during resealing to attain as per above final concentrations.

Loading by red cell loader:[12-14]

Magnani and coworkers, 1998 developed a novel method for the entrapment of non diffusible drugs into human erythrocytes. The equipment designed for this method was termed as "red cell loader". The method requires as little as 50ml of blood.

Hypotonic dilution or Dilution method: [15,16]

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. The major drawbacks of this method include low entrapment efficiency and a considerable loss of hemoglobin and other cell components. This reduces the circulation half life of the loaded cells. These cells are readily phagocytosed by RES macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as B-galactosidase and B-glycosidase, asparginase, and arginase, as well as bronchodilators such as salbutamol.

Hypotonic pre-swelling: [17,18]

The technique is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low values. The supernatant is discarded and the cell fraction is brought to the lysis point by an aqueous solution of the drug to be encapsulated. Adding 100–120. The mixture is centrifuged between the drug-addition steps. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer

Entrapment by endocytosis: [19]

Endocytosis involves the addition of one volume of washed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5 mM MgCl2, and 1mM CaCl2, followed by incubation for 2 min at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37oC for 2 min. The entrapment of material occurs by endocytosis .The vesicle membrane separates endocytosed material from cytoplasm thus protecting it from the erythrocytes and vice-versa.

Loading by electric cell fusion: [20]

This method involves the initial loading of drug molecules into erythrocyte ghosts followed by adhesion of these cells to target cells. The fusion is accentuated by the application of an electric pulse, which causes the release of an entrapped molecule. An example of this method is loading a cell specific monoclonal antibody into an erythrocyte ghost.

Loading by lipid fusion: [21]

Lipid vesicles containing a drug can be directly fused to human erythrocytes, which lead to an exchange with a lipid-entrapped drug. This technique was used for entrapping inositol monophosphate to improve the oxygen carrying capacity of cells. However, the entrapment efficiency of this method is very low (~1%).

APPLICATIONS OF RESEALED ERYTHROCYTES:

Resealed erythrocytes have several possible applications in various fields of human and veterinary medicine. Such cells could be used as circulating carriers to disseminate a drug

within a prolonged period of time in circulation or in target-specific organs, including the liver, spleen, and lymph nodes.

Slow drug release:

Erythrocytes have been used as circulating depots for the sustained delivery of antineoplastics antiparasitics, veterinary antiamoebics, vitamins, steroids antibiotics, and cardiovascular drugs. The various mechanisms proposed for drug release include-

- Passive diffusion
- Specialized membrane associated carrier transport
- Phagocytosis of resealed cells by macrophages of RES, subsequent accumulation of drug into the macrophage interior, followed by slow release.
- Accumulation of erythrocytes in lymph nodes upon subcutaneous administration followed by hemolysis to release the drug.[28]

Targeting to the liver:

Many metabolic disorders related to deficient or missing enzymes can be treated by injecting these enzymes. However, the problems of exogenous enzyme therapy include a shorter circulation half life of enzymes, allergic reactions, and toxic manifestations. These problems can be successfully overcome by administering the enzymes as resealed erythrocytes. The enzymes used include β-glycosidase, β-glucoronidase, and β-galactosidase.[29]

Targeting of bioactive agents to RES:

After drug release, remnants of erythrocytes are rapidly cleared from circulation by phagocytosis. Targeting of the drug minimizes its side effects and the dose to be administered as well as drug utilization. Modifications of erythrocytes membranes by treating them with antibodies, gluteraldehyde, sialic acid, ascorbate, ferrous ion, biotin and sulfhydryl containing substances accelerate their targeting to the liver as well as spleen.[30]

Removal of toxic agents:

Cannon *et al*. reported inhibition of cyanide intoxication with murine carrier erythrocytes Containing bovine rhodanase and sodium thiosulfate. Antagonization of organophosphorus Intoxication by resealed erythrocytes containing a recombinant phosphodiestrase also has been reported.[31]

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. Nucleosides are rapidly transported across the membrane whereas

Nucleotides are not and thus exhibiting prolonged release profiles. The release of nucleotides requires conversion of these moieties to purine or pyrimidine bases. Resealed erythrocytes have been used to deliver deoxycytidine derivatives, recombinant herpes simplex virus type1 glycoprotein B, azidothymidine derivatives, azathioprene, acyclovir, and fludarabine phosphate.[32]

Enzyme therapy: [33-35]

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

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. The use of resealed erythrocytes looks promising for a safe and sure delivery of various drugs for passive and

active targeting. In coming future, erythrocytes based delivery system with their capability to afford controlled and site specific drug delivery have been developed for disease management. Erythrocyte carriers are **"Nano device in field of Nanotechnology**". In this respect this review work definitely be profitable for us and for all those researchers who are intentionally involve in research work of drug carrier

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