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A Review on Resealed Erythrocyte as a Newer Drug Carrier System

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ABSTRACT

Cellular carriers meet several desirable criteria in clinical applications, among the most important being the carrier's biocompatibility and its degradation products, the various carriers used to target drugs to different body tissues. Among these, the most studied erythrocytes have been shown to have greater potential in the delivery of drugs here are currently 30 major products on the market to deliver drug. Carrier erythrocytes have been evaluated for the safety and efficacy of treatments in thousands of human drug administration. Because of their remarkable degree of biocompatibility, biodegradability and a number of other potential benefits, carrier erythrocytes, resealed erythrocytes charged by a drug or other therapeutic agents have been extensively exploited in recent years to provide a wide range of drugs and other bioactive agents temporally and spatially controlled. Biopharmaceuticals, therapeutically significant peptides and proteins, biological, antigens, and nucleic acid-based vaccines are among the recently targeted drugs for delivery using erythrocyte carriers. Carrier erythrocytes are prepared by collecting blood samples from the organism of intreast and separating plasma erythrocytes. The cells are separated by different methods and the product is retained in the erythrocytes, they are eventually resealed and the resulting carriers are then labeled "resealed erythrocytes". Recent research focuses on isolation, methods of drug processing, testing methods and drug delivery resealed erythrocyte applications.

Keywords: *Characterization methods, drug targeting, isolation and applications, resealed erythrocytes*

INTRODUCTION

Blood contains various types of cells such as erythrocytes (RBC), leucocytes (WBC) and platelets, including erythrocytes, which are the most interesting carrier and possess great potential for drug delivery

due to their ability to circulate throughout the body, zero-order kinetics, reproducibility and easy preparation. [1]. The overall process is based under osmotic conditions on the response of these cells. The drug-charged erythrocytes function as slow circulating depots after reinjection and direct the drugs to tissue or organ disease. The objective of the current pharmaceutical scenario is to construct drug delivery systems that optimize drug targeting along with high therapeutic benefits for safe and effective management of disease. Targeting an active bio molecule from the successful delivery of drugs where the pharmacological agent specifically targets its target site. Drug targeting can be either by chemical modification or by a suitable carrier.

Erythrocytes

Red blood cells (also known as erythrocytes) are the most common type of blood cells and the main means of delivering oxygen (O_2) through the circulatory system to the body tissues through the blood flow. In the bone marrow, the cells mature and circulate in the body for about 100–120 days until macrophages reuse their components. It takes about 20 seconds for each circulation. About a quarter of the human body's cells are red blood cells [2].

Anatomy, physiology and composition of RBC's

RBC's have shapes such as 7.8 μm diameter biconcave disks and about 2.2 μm thickness. There is a simple structure for mature RBCs. By nature, it's also elastic. Their plasma membrane is both strong and flexible, allowing them to squeeze through narrow capillaries without rupturing. RBCs lack a nucleus and other organelles and are unable to replicate or perform extensive metabolic activity. For their oxygen transport function, RBCs are highly specialized because their mature RBCs do not have a nucleus, and all their internal space is available for oxygen transport. Even the design of the RBC equipment it operates [13]. For the movement of gas molecules into and out of the RBC, a biconcave disk has a much larger surface area than it would; say a sphere or cube. The red blood cell membrane, a complex, semi-permeable cell element associated with energy metabolism in maintaining the cell's permeability characteristics of different cations (Na+, K++) and anions (Cl-HCO-). Each RBC contains about 280 million hemoglobin molecules. A hemoglobin molecules consists of a protein called globin, composed of four polypeptide chains; each of the four chains is bound to a non-protein-like pigment called a heme. Combine reversibly with one oxygen molecule at the center of the heme chain so that each hemoglobin molecule binds four oxygen molecules. RBCs include water (63%), lipids (0.5%), glucose (0.8%), minerals (0.7%), non-hemoglobin (0.9%), meth hemoglobin (0.5%) and hemoglobin (33.67%).

Resealed Erythrocytes

The drug-loaded carrier erythrocytes are prepared simply by collecting blood samples from the interesting organism, separating erythrocytes from plasma, trapping drugs in the erythrocytes, and resealing the resulting cell carriers. Such carriers are therefore referred to as resealed erythrocytes [3]. The overall process is based under osmotic conditions on the response of these cells. The drug-charged erythrocytes act as slow circulating depots after reinjection and target the drugs to a reticuloendothelial system (RES).

Source of Erythrocytes

Various types of mammalian erythrocytes, including mice, cattle, pigs, dogs, sheep, goats, monkeys, chicken, rats, and rabbits, have been used for drug delivery.

Isolation of Erythrocytes

Erythrocytes can be prepared as carriers of human and animal blood, such as rodents, mice, rabbits, cats, etc. Blood is obtained from the corresponding human, mouse, rodent, or animal species using an effective anti-coagulant. EDTA is typically used as an anticoagulant that better protects the properties of blood cells. In a refrigerated centrifuge, freshly collected blood is centrifuged to isolate packed erythrocytes [11]. Subsequently, several washes are performed. This is a process that usually involves repeated centrifugation with a solution for removing other components of the blood. By using a capillary hollow fibre plasma separator, the erythrocytes can be washed more efficiently. The hematocrits employed can vary from 5% to 95% [6]. Although the most common thing is to work with a 70% hematocrit. In 1953, using adenosine triphosphate (ATP), Gardos tried to load erythrocyte ghost. In 1959, dextran (molecular weight 10–250 kDa) was reported by Marsden and Ostting. In 1973, Ihler et al. and Zimmermann separately reported the loading of drugs into erythrocytes. In 1979, to characterize drug-loaded erythrocytes, the term carrier erythrocytes was coined.

Requirement for Encapsulation

Biologically active substance variety (5000-60,000dalton) may be trapped in erythrocytes.

- Non-polar molecule may be trapped in salt erythrocytes. Example: tetracycline HCl salt in bovine RBC can be significantly trapped [12].
- Generally speaking, the molecule should also be trapped with Polar & Non Polar molecule.
- Once the charged molecule is encapsulated, it will hold longer than the unloaded molecule. When the molecule is smaller than sucrose and larger than B-galactosidase, the size of the trapped molecule is a significant factor.
- Various condition and centrifugal force used for isolation of erythrocytes.

METHODS OF DRUG LOADING IN ERYTHROCYTES

Hypo-osmosislysis Method

In this process, osmotic lysis and resealing is exchanged for the intracellular and extracellular solution of erythrocytes. This process will encapsulate the drug present in the RBCs.

Hypotonic Dilution

A amount of packed erythrocytes is combined in this process with 2–20 volumes of a drug's aqueous solution. Then a hypertonic buffer is added to restore the tonicity of the solution. The resulting mixture is then centrifuged, the supernatant is discarded, and the isotonic buffer solution washes the pellet.

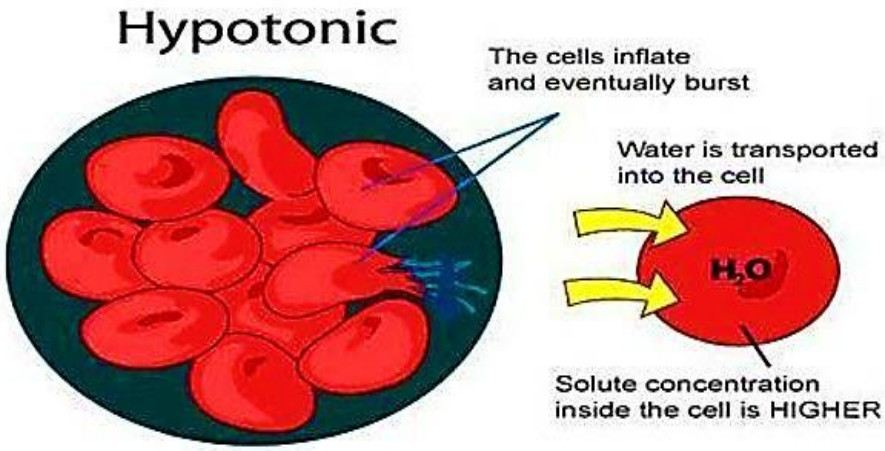

Figure 1: *Hypotonic dilution.*

Hypotonic Dialysis Method

Klibansky first reported this method in 1959 and Deloach, Ihler and Dale used this method in 1977 to load enzymes and lipids. In this process, an isotonic, buffered erythrocyte suspension with a hematocrit value of 70–80 is prepared and placed in a conventional 10–20 volume dialysis tube of a hypotonic buffer [4]. The medium is steadily disturbed for 2 hours. The tonicity of the dialysis tube is restored by adding directly to the surrounding medium a calculated amount of a hypertonic buffer or by replacing the surrounding medium with an isotonic buffer. The drug to be loaded can be added either by dissolving the drug inside a dialysis bag at the beginning of the experiment in an isotonic cell suspending buffer or by adding the drug to a dialysis bag after the stirring is complete.

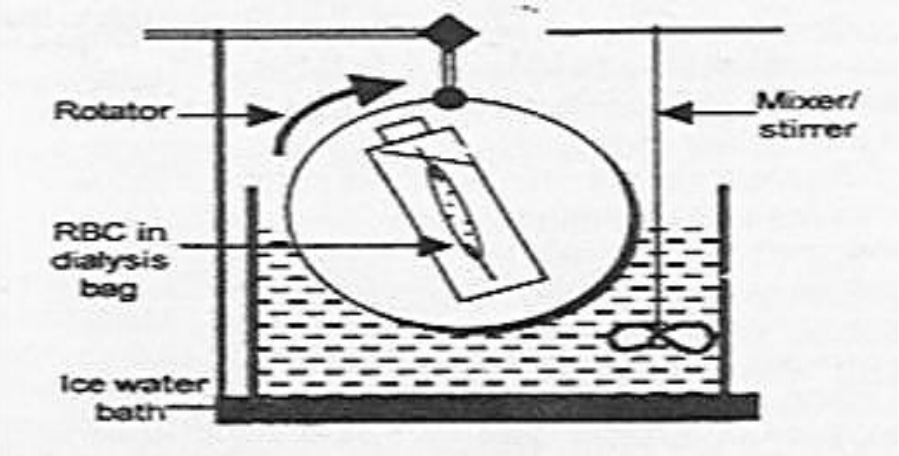

Figure 2: *Hypotonic dialysis.*

Hypotonic Press Welling Method

Rechsteiner invented this approach in 1975 and was updated for drug charging by Jenner et al. By positioning them in a slightly hypotonic solution, this approach is based on the principle of first swelling the erythrocytes before lysis. The swollen cells are recovered at low speed through centrifugation. Instead relatively small amounts are added to the point of lysis of aqueous drug solution. The slow swelling of cells leads to good preservation of cytoplasmic components and therefore good in vivo survival. Simpler and quicker than other approaches, this approach causes minimal damage to cells. This method includes propranolol, asparginase, cyclopohphamide, methotrexate, insulin, metronidazole, levothyroxine, enalaprilate & isoniazid.

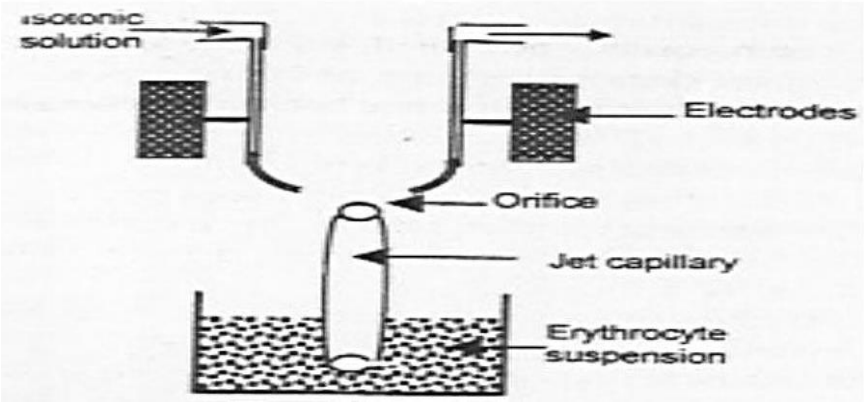

Figure 3: *Hypotonic press well method.*

Isotonic Osmotic Lysis Method

This method was documented by Schrier et al., in 1975. This technique, also known as the osmotic pulse process, involves isotonic hemolysis by physical or chemical means. It may or may not be solutions that are isotonic. Because of the gradient of concentration, if erythrocytes are incubated in the solutions of a substance with high membrane permeability, the solution will diffuse into the cells.Following this process is an influx of water to maintain osmotic balance. Of isotonic hemolysis, chemicals such as urea solution, polyethylene glycol, and ammonium chloride were used. This method is also not immune to changes in the composition of the membrane structure, however. Franco et al., developed a method in 1987 involving the suspension of erythrocytes in a dimethyl sulfoxide (DMSO) isotonic solution. The suspension was combined with a drug solution isotonically buffered. They were sealed at 370°C after the cells had been separated (Fig. 4).

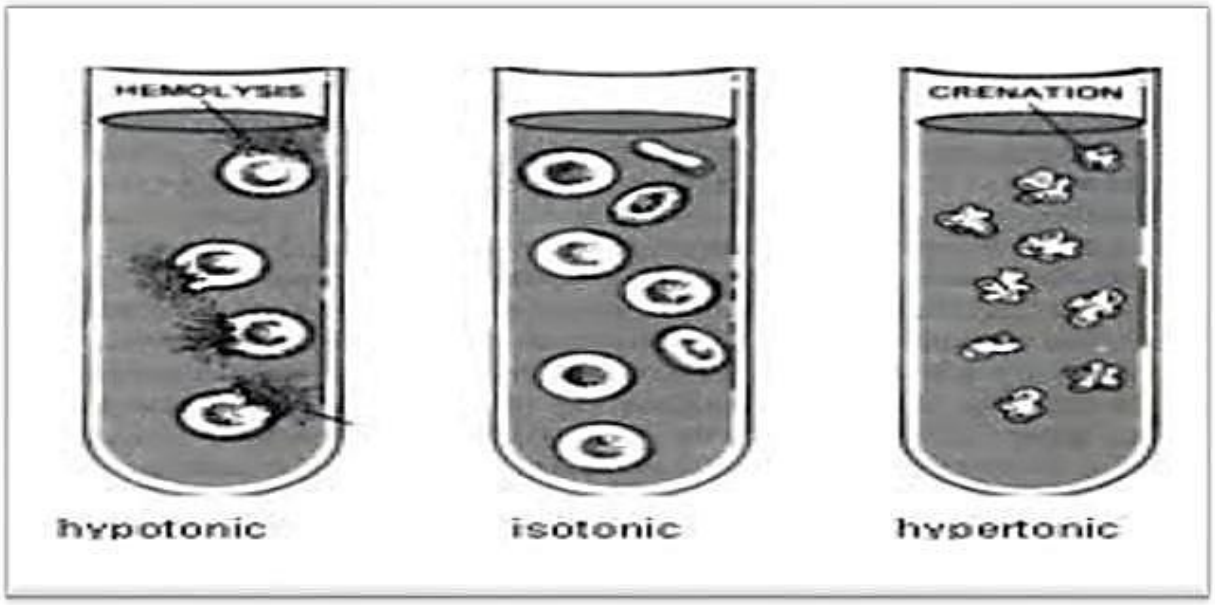

Figure 4: *Isotonic osmotic lysis.*

Electro-insertion or Electro Encapsulation Method

In 1973, Zimmermann attempted to encapsulate bioactive molecules using an electrical pulse technique. Also known as electroporation, the method is based on the observation of irreversible changes in an erythrocyte membrane caused by electrical shock. Also called this method as an electroporation. Erythrocyte membrane is opened by a dielectric breakdown in this method; erythrocyte pore can subsequently be resealed in an isotonic medium at 370°C by incubation. Primaquine and related 8-amino quinolone, vinblastin chloropromazine and related phenothiazine, propanolol, tetracaine, and vitamin A are the different chemicals encapsulated in the erythrocytes.

Entrapment by Endocytosis

Schrier et al reported this method in 1975. This method involves adding one volume of washed packed erythrocytes to nine volumes of 2.5MM ATP, 2.5MM mgcl2 and 1MM CaCl2 buffer, followed by incubation at room temperature for 2 minutes. By using NaCl 154MM, the pores created by this method are resealed and incubated for 2 minutes at 370°C. This method involves primaquine and related8-aminoquinoline, vinblastin, chlorpromazine, and related phenothiazines, hydrocortisone, tetracaine, and vitamin A.

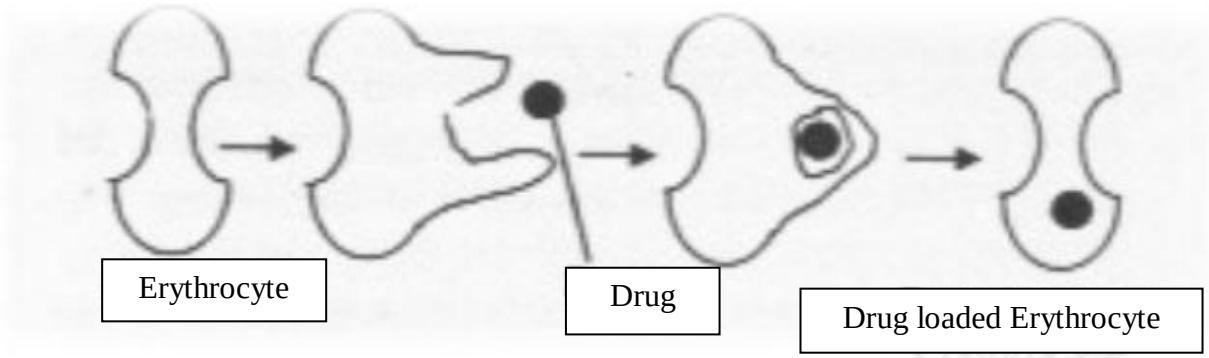

Figure 5: *Entrapment by endocytosis.*

Chemical Perturbation of the Membrane

This approach is based on the increase in erythrocyte membrane permeability when exposed to certain chemicals by the cells. In 1973, Deuticke et al. showed that when exposed to polyene antibiotics such as amphotericin B, the permeability of erythrocyte membrane increases. In 1980, this approach was successfully used in human and mouse erythrocytes by Kitao and Hattori to capture the antineoplastic drug daunomycin [5]. These methods, however, induce irreversible destructive changes in the membrane of the cells and are therefore not very popular.

Lipid Fusion Method

The lipid vesicles containing a drug may be fused directly to human erythrocytes, resulting in an exchange with a drug trapped in lipid 161. The methods are useful for clogging monophasphate inositol to improve cell oxygen carrying capacity and this method's clogging efficiency is very low (~1%).

Loading by Electric Cell Fusion

This method involves the initial loading into erythrocyte ghosts of drug molecules accompanied by adherence of these cells to target cells. Applying an electrical pulse, which causes the release of a trapped molecule, accentuates the fusion. An example of this approach is the loading into an erythrocyte ghost of a cell-specific monoclonal antibody. An antibody against a specific targetcell surface protein may be chemically linked to drug-charged cells that would guide these cells to targeted cells.

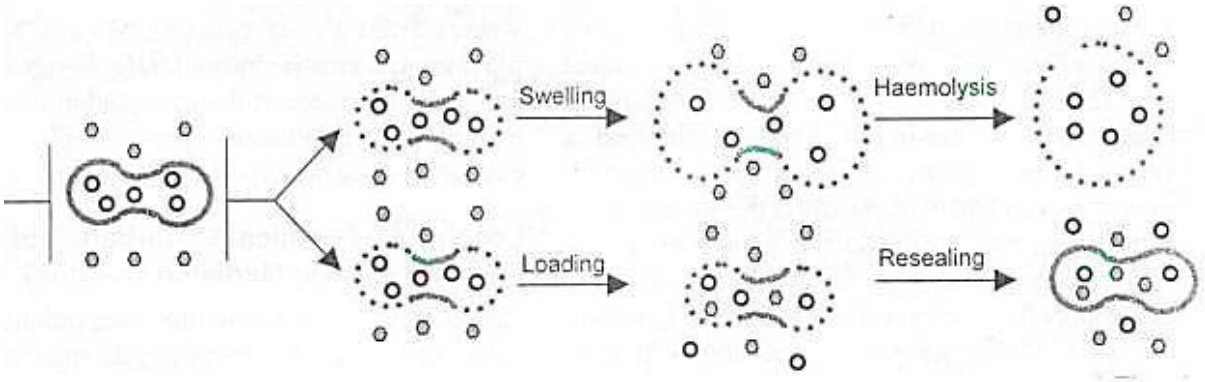

Figure 6: *Loading by electric cell fusion.*

Use of Red Cell Loader

Novel method for trapping non-diffusible drugs in erythrocytes has been established. A piece of equipment called a "red cell loader" was created. With as little as 50 ml of a blood sample, under blood banking conditions, various biologically active compounds were caught in erythrocytes

within 2 h at room temperature. The process is based on two simultaneous hypotonic dilutions of washed erythrocytes followed by a hem filter concentration and isotonic cell resealing. For 35–50 per cent cell regeneration, there was 30% drug charging. The erythrocytes that were treated had natural in vivo survival. Through enhancing their identification by tissue macrophages, the same cells could be used for targeting.

STORAGE

Store encapsulated preparation without loss of integrity when suspended in hank's balanced salt solution [HBSS] at 4°C for two weeks. Use of group 'O' [universal donor] cells and by using the preswell or dialysis technique, batches of blood for transfusion. Standard blood bag may be used for both encapsulation and storage [14].

APPLICATIONS OF RESEALED ERYTHROCYTES

In-Vitro Application

To order to facilitate the absorption of enzymes, phagocytosis cells were used to *in vitro*. Using cytochemical technique, enzymes within carrier RBC could be visualized. Deficiencies such as the deficiency of glucose-6-phosphate dehydrogenase (G6PD) may be a useful tool to discern the mechanism that ultimately causes these effects. RBC's most commonly used in vitro is microinjection. A fusion process injected a protein or nucleic acid into eukaryotic cells [6]. Likewise, they propagate rapidly throughout the cytoplasm when antibody molecules are added using erythrocytic carrier method. Auto-injected RBC antibody into living cells was used to validate the toxin fragment's site of action. Antibodies introduced using RBC mediated microinjection are recorded to prevent entry into the nucleus, limiting the studies to cytoplasmic level. *In-Vivo* Application:

Targeting of Bioactive Agents to RE System

The phagocyte Kuffer cells in the liver and spleen quickly clear up damaged erythrocytes from circulation. Thus, resalted erythrocytes can be used to target the liver and spleen by modifying their membranes. The different approaches to modifying erythrocyte surface characteristics include surface modification with antibodies, gluteraldehyde, carbohydrates such as sialic acid and sulphydryl.

Targeting to Sites as Other Than RES Organ

Resealed erythrocytes have the ability to supply the macrophage-rich with a drug or enzyme. Resealed erythrocytes have recently been used to test organs targeting other than RES. Some of the approaches to representation are discussed briefly.

Targeting the Liver

Deficiency / replacement of enzyme therapy. Through administering these enzymes, most metabolic conditions associated with defective or absent enzymes can be controlled. Exogenous enzyme therapy problems, however, include shorter enzyme distribution, allergic reactions, and are toxic [7]. The enzymes used include glycosidase, glucuronidase, and galactosidase4, which can be successfully solved by administering the enzymes as resealed. Glucocerebrosi-dase-loaded erythrocytes can treat the disease caused by an accumulation of glucocerebroside in the liver and spleen [3].

Slow Drug Release

Erythrocytes have been used as circulating depots to provide antineoplastics, antiparasitic, veterinary antiamoebics, vitamins, hormones, antibiotics and cardiac drugs on an ongoing basis

Drug Targeting

Ideally, in order to achieve optimum therapeutic index with minimal adverse effects, drug delivery should be site-specific and target driven. Resealed erythrocytes can also function as drug carriers and target devices. Modified surface erythrocytes are used to target mononuclear phagocytic system / RES organs because macrophages recognize the change in the membrane.

Targeting Liver-Deficiency/Therapy

Through administering these enzymes, most metabolic disorders associated with defective or absent enzymes can be treated [15]. However, problems with exogenous enzyme therapy include shorter half-life enzyme circulation, allergic reactions, and toxic manifestations. By administering resealed erythrocytes to the enzymes, these problems can be successfully overcome. P-glucosidase, P-glucoronidase, and P-galactosidase are the enzymes that are used. Glucocerebrosidase-load d erythrocytes may treat the disease caused by an accumulation of glucocerebroside in the liver and spleen.

Removal Toxic Agents

Inhibition of cyanide poisoning with murine carrier erythrocyte containing bovine rhodanase and sodium thiosulphate was reported by Cannon et al. It has also been reported that organophosphorus poisoning has been antagonized by released erythrocyte with recombinate phosphodiestrase.

ROUTE OF ADMINISTRATION

The intra-peritoneal injection was stated to be similar to the cells administered by i.v. Injection. They reported that 25% of the resealed cell remained in circulation for 14 days as a method for extra-vascular targeting of RBCs to peritoneal macrophages. Subcutaneous way to release trapped agents slowly [8]. They reported that encapsulated molecules were released at the injection site by the loaded cell.

EVALUATION OF RESEALED ERYTHROCYTES

Shape and Surface Morphology

After administration, the morphology of erythrocytes determines their life span. In comparison to untreated erythrocytes, the morphological characterization of erythrocytes is conducted using either transmission (TEM) or scanning electron microscopy (SEM). Certain techniques, such as phase contrast microscopy, can also be used [9].

Drug Content

Cell drug content determines the effectiveness of the method used in trapping. The process involves deproteinizing sealed, packed cells (0.5 mL) with 2.0 mL acetonitrile and 10 min at 2500 rpm centrifugation. Spectrophotometrically, the clear supernatant is analyzed for the drug content.

Cell Counting and Cell Recovery

This includes counting the number of red blood cells per unit volume of whole blood, which is typically calculated by counting the number of intact cells per cubic mm of packed erythrocytes before and after the medication is loaded using an automated system.

Turbulence Fragility

It is determined by passing cell suspension through smaller inner diameter needles (e.g. 30 gauges) or shaking the cell suspension vigorously[10]. In both cases, after the process is determined, haemoglobin and medication released. Resealed cells ' chaotic fragility is found to be higher.

Erythrocyte Sedimentation Rate

It is an approximation of RBC's suspension stability in plasma and is related to the number and size of red cells and related plasma protein concentration, especially fibrinogen and α, β globulins.

This experiment is carried out by evaluating the sedimentation level in a typical pipe of blood cells. Normal ESR blood is between 0 and 15 mm / h. Indications of active but obscure disease processes are higher rates.

Determination of Entrapped Magnetite

For determining the concentration of specific metal in the sample, atomic absorption spectroscopic method is reported. Using atomic absorption spectroscopy, the HCl is added to a fixed amount of magnetite bearing erythrocytes and content is heated at 600C for 2 hours, then 20% w / v trichloro acetic acid is added and supernatant is obtained after centrifugation.

Haemoglobin Release

During the encapsulation process, the haemoglobin content of the erythrocytes may be decreased by changes in the permeability of the red blood cell membrane. In addition, the relationship between the haemoglobin concentration and the drug release rate of the erythrocyte-encapsulated material. The leakage of haemoglobin is checked with a red cell suspension through absorption

In-vitro Drug Release and Hb Content

The cell suspensions are stored at 40°C in ambered glass container (5% hematocrit in PBS). Periodically clear supernatants are drawn using a 0.45 filtered hypodermic syringe, deprotected using methanol, and far-reaching drug content has been estimated. The supernatant of each sample can be measured using formula percent Hb release= A540 of 100% Hb history test-A540 after centrifugation collected and assayed [16-19].

CONCLUSION

The use of resealed erythrocytes has a very good result for a safe and secure distribution for passive and active targeting of different drugs. The concept requires more refinement in order to become a standard drug delivery method and we can also use the same principle to expand the delivery of biopharmaceuticals and much remains to be explored as to the ability of resealed erythrocytes. It's very easy to prepare resealed erythrocytes. Now a day has been identified with several strategies that we can quickly trap the drug into erythrocytes. It has both *in-vitro* and *in-vivo* form of application.

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