Erythrocytes as Drug Delivery System: A Boon to Cure

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Abstract

The biocompatibility, non-immunogenicity, non-pathogenicity and biodegradability make the erythrocytes as unique and useful carriers. Erythrocytes are non-toxic and biocompatible with human body. The specific and accurate amount of drug will be incorporated into the cells by causing osmotic manipulation in cell structure. Biopharmaceuticals, therapeutically significant peptides and proteins, nucleic acid-based biological, antigens, anticancer drug and Vaccines are among the recently focused pharmaceuticals for being delivered using carrier Erythrocytes. ***So*** *many drugs like aspirin, steroid, cancer drug which have many side effects are reduced by use of resealed Erythrocytes. Current review highlights isolation, drug loading methods, evaluation methods and applications of resealed Erythrocytes for drug delivery.*

Keywords: Erythrocytes, Drug Delivery System, Manipulation, Biopharmaceutical.

1. Introduction

Blood contains different type of cells like Erythrocytes (RBC), Leucocytes (WBC) and Platelets. Among them, Erythrocytes are the most interesting carrier and possess great potential in drug delivery due to their ability to circulate throughout the body, zero order kinetics, reproducibility and ease of preparation. Primary aim for the development of this drug delivery system is to maximize therapeutic performance, reducing undesirable side effects of drug as well as increase patient compliance [9]. The overall process is based on the response of these cells under osmotic conditions. Upon reinjection, the drug-loaded erythrocytes serve as slow circulating depots and target the drugs to disease tissue or organ. Present pharmaceutical scenario is aimed at development of drug delivery systems which maximize the drug targeting along with high therapeutic benefits for safe and effective management of diseases. Targeting of an active bio molecule from effective drug delivery where pharmacological agent directed specifically to its target site. Drug targeting can be done by approaches as either chemical modification or by appropriate carrier.

2. 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 (O_2) to the body tissues via the blood flow through the circulatory system. The cells develop in the bone marrow and circulate for about 100–120 days in the body before their components are recycled by macrophages. Each circulation takes about 20 seconds. Approximately, quarters of the cells in the human body are red blood cells.

3. Physiology

Red blood cells are highly specialized for their oxygen transport function. Because mature RBCs have no nucleus, all their internal space is available for oxygen transport. Because RBCs lack mitochondria and generate ATP an aerobically (without oxygen), they do not use up any of the oxygen they transport. Even the shape of RBC facilitates its function. A biconcave disc has a

much greater surface area for the diffusion of gas molecules in to and out of the RBC than would, say, a sphere or a cube. Each RBC contains about 280 million hemoglobin molecules.

A hemoglobin molecule consists of a protein called **globin,** composed of four polypeptide chains (two alpha and two beta chains); a ring like non protein pigment called a **heme** (Figure 1) is bound to each of the four chains. At the center of eachheme ring is an iron ion that can combine reversibly with one oxygen molecule (Figure 1), allowing each hemoglobin molecule to bind four oxygen molecules. Each oxygen molecule picked up from the lungs is bound to an iron ion. As blood flows through tissue capillaries, the iron–oxygen reaction reverses. Hemoglobin releases oxygen, which diffuses first into the interstitial fluid and then into cells.

Hemoglobin also transports about 23% of the total carbon dioxide, a waste product of metabolism. Blood flowing through tissue capillaries picks up carbon dioxide, some of which combines with amino acids in the globin part of hemoglobin. As blood flows through the lungs, the carbon dioxide is released from hemoglobin and then exhaled. In addition to its key role in transporting oxygen and carbon dioxide, hemoglobin also plays a role in the regulation of blood flow and blood pressure.

The gaseous hormone **Nitric oxide (NO),** produced by the endothelial cells that line blood vessels, binds to hemoglobin. Under some circumstances, hemoglobin releases NO. The released NO causes *vasodilatation,* an increase in blood vessel diameter that occurs when the smooth muscle in the vessel wall relaxes. Vasodilation improves blood flow and enhances oxygen delivery to cells near the site of NO release.

4. Source of Erythrocytes

Various types of mammalian Erythrocytes have been used for drug delivery, including erythrocytes of mice, cattle, pig, dog, sheep, goat, monkey, chicken, rat, and rabbit.

5. Isolation of Erythrocytes

Erythrocytes may be prepared as carriers from blood taken from human beings and from different animal species, such as rat, mice, rabbit, dog, etc. [8]. Blood is taken from the human being, mouse, rat or the animal species in question, using a suitable anti-coagulant. Application is normally made of EDTA, as it is the anticoagulant that best preserves the properties of blood cells. Freshly collected blood is centrifuged in a refrigerated centrifuge in order to separate packed Erythrocytes. Several washes are subsequently performed. This is a process that normally involves repeated centrifugation with an isosmotic solution to remove other blood components. The Erythrocytes can be washed more efficiently by using a capillary hollow fiber plasma separator. The hematocrits employed may be variables ranging between 5 % and 95 %, although the most usual is to work with a hematocrit of 70 %. In 1953, Gardos tried to load Erythrocyte ghost using Adenosine triphosphate (ATP). In 1959, Marsden and Ostting reported the entrapment of dextran (molecular weight 10–250 kDa). In 1973, the loading of drugs in Erythrocytes was reported separately by Ihler et al. and Zimmermann. In 1979, the term carrier Erythrocytes was coined to describe drug-loaded Erythrocytes [1], [3], [7].

6. Methods of Drug Loading

Several methods have been reported for encapsulation of drug or other bioactive agents in Erythrocytes. Some of these methods such as electrical pulse methods and osmosis-based methods have a physical nature whereas the other methods such as the chemical perturbation of the

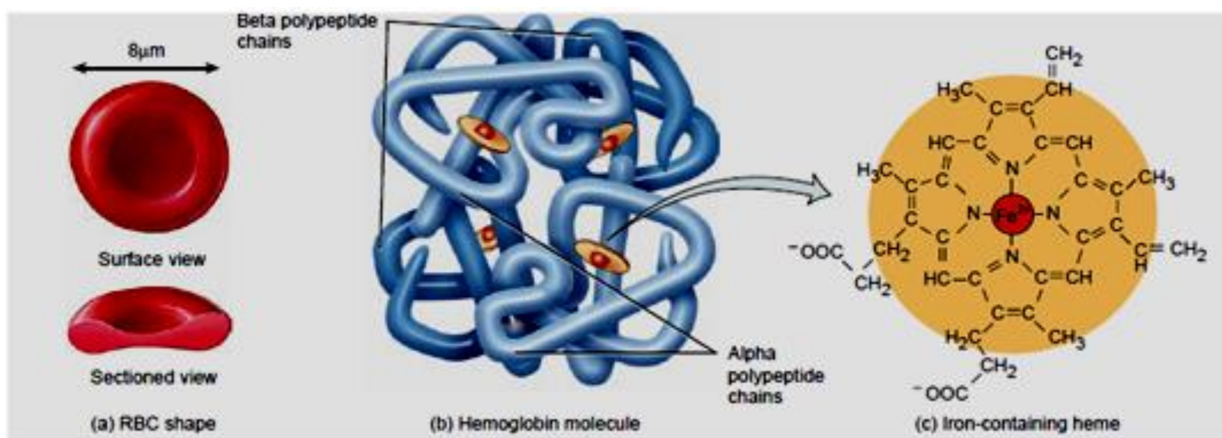

Figure 1

membrane are chemically based. Regardless of the method used, the optimal characteristics for a compound to be encapsulated successfully in Erythrocytes include a considerable degree of water solubility, resistance against inactivation within the Erythrocytes, the lack of physical and/or chemical interaction with Erythrocyte membrane or the other cell constituents, and well-defined pharmacokinetic as well as pharmaco-dynamics properties. Hypotonic hemolysis, hypotonic dilution, hypotonic dialysis, hypotonic pre swelling, and osmotic pulse methods are categorized as osmosis-based methods. Chemical perturbation of the membrane, electrical breakdown or 'electroporation', endocytosis, lipid fusion, laser loading, and intrinsic uptake of substances by Erythrocytes are other reported methods used for encapsulation of drugs and other agents into Erythrocytes.

7. Hypotonic Hemolysis

This method is based on the ability of Erythrocytes to undergo reversible swelling in a hypotonic solution. Erythrocytes have an exceptional capability for reversible shape changes with or without accompanying volume change and for reversible deformation under stress. An increase in volume leads to an initial change in the shape from biconcave to spherical. This change is attributable to the absence of superfluous membrane; hence the surface area of the cell is fixed. The cells assume a spherical shape to accommodate additional volume while keeping the surface area constant. The volume gain is 25–50 %. The cells can maintain their integrity up to a tonicity of ~150 moss/kg, above which the membrane ruptures, releasing the cellular contents. At this point (just before cell lysis), some transient pores of 200–500 Å are generated on the membrane. After cell lysis, cellular contents are depleted. The remnant is called an Erythrocyte ghost. The principle of using these ruptured Erythrocytes as drug carriers is based on the fact that the ruptured membranes can be resealed by restoring isotonic conditions. Upon incubation, the cells resume their original biconcave shape and recover original impermeability.

8. Use of Red Cell Loader

A novel method for entrapment of non-diffusible drugs into Erythrocytes is by red cell loader method. With as little as 50 ml of a blood sample, different biologically active compounds were entrapped into Erythrocytes within a period of 2 hours at room temperature under blood banking conditions. The process is based on two sequential hypotonic dilutions of washed Erythrocytes followed by concentration with a hem filter and an isotonic resealing of the cells. There was ~30 % drug loading with 35-50 % cell recovery. The processed Erythrocytes had normal survival in vivo. The same cells could be used for targeting by improving their recognition by tissue macrophages.

9. Hypotonic Dilution

Hypotonic dilution was the first method investigated for the encapsulation of chemicals into Erythrocytes and is the simplest and fastest method. 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 phagocytosis by RES (Reticulo-endothelial system) macrophages and hence can be used for targeting RES organs. Hypotonic dilution is used for loading enzymes such as β-galactosidase and β-glucosidase, asparginase and arginase, as well as bronchodilators such as salbutamol.

10. Hypotonic Pre-swelling

This method was developed by Rechsteinerin in 1975 and was modified by Jenner et al. for drug loading. 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 µl portions of an aqueous solution of the drug to be encapsulated. The mixture is centrifuged between the drug-addition steps [10]. 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. Then, the cell suspension is incubated at 37° C to reanneal the resealed Erythrocytes. Such cells have a circulation half-life comparable to that of normal cells [6], [11]. This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in Erythrocytes using this method include propranolol, asparginase, cyclopohphamide, cortisol-21-phosphate, ώ1-antitrypsin, metronidazole, levothyroxine, methotrexate, insulin, enalaprilat and isoniazid [8].

11. Hypotonic dialysis

This method was first reported by Klibanskyin 1959 and was used in 1977 by Deloach and Ihler, and Dale for loading enzymes and lipids [2]. 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 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 [4], [10]. The medium is agitated slowly for 2 hours [4]. 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 [7].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. The use of standard hemodialysis equipment for loading a drug in Erythrocytes was reported by Roper et al. In this method, the Erythrocyte suspension and the drug to be loaded were placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment [5], [6], and [9]. This led to the concept of “continuous flow dialysis,” which has been used by several other researchers. The loaded cells exhibit the same circulation half life as that of normal cells. Also, this method has high entrapment efficiency on the order of 30-50 %, cell recovery of 70-80 %, high-loading capacity, and is amenable to automation with control of process variables. The drawbacks include a long processing time and the need for special equipment [7], [10]. This method has been used for loading enzymes such as β-galactosidase, glucoserebrosidase , asparginase, inositol hexaphosphatase, as well as drugs such as gentamicin, Adriamycin,pentamidine and furamycin, interlukin-2, desferroxamine , and human recombinant erythropoietin [2], [5], [11].

12. Isotonic Osmoticlysis

This method, also known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means [2], [4]. The isotonic solutions may or may not be isoionic. 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 [1], [3], [7]. However, this method also is not immune to changes in membrane structure composition. In 1987, Franco et al. developed a method that involved suspending Erythrocytes in an isotonic solution of Dimethyl sulfoxide (DMSO).The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37°C [10].

13. 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 et al. showed that the permeability of Erythrocytic membrane increases upon exposure to polyene antibiotic such as amphotericin B. In 1980, this method was used successfully by Kitao and Hattori to entrap the antineoplastic drug daunomycin in human and mouse Erythrocytes. Lin et al. used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

14. 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. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading. 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 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. Various compounds such as sucrose, urease, methotrexate, isoniazid, human glycophorin, DNA fragments, and latex particles of diameter 0.2 μm can be entrapped within Erythrocytes by this method. Mangal and Kaur achieved sustained release of a drug entrapped in Erythrocytes with the use of electroporation.

15. Entrapment by Endocytosis

This method was reported by Schrier et al. in 1975. Endocytosis involves the addition of one volume of washed packed Erythrocytes to nine volumes of buffer containing 2.5 mm ATP, 2.5 mm $MgCl_2$, 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 Na Cl and incubation at 37°C for 2 min.

16. Loading by Electric Cell Fusion

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. An antibody against a specific surface protein of target cells can be chemically cross-linked to drug-loaded cells that would direct these cells to desired cells.

17. Loading by lipid fusion

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 %).

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