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Review Article

Resealed Erythrocyte: An Updated Engineering and Novel Approach for Targeted Drug Delivery

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Abstract

Erythrocytes or red blood cells, has been extensively studied for their capability as a potential carrier for the targeted drug delivery. Resealed erythrocytes, as a targeted drug delivery system has excellent capacity to enhance the bio-availability, therapeutic index and patient compliance. They are popular because they are bio-compatible, biodegradable, possess longer circulation half-life, show zero order drug release kinetics and can be loaded with variety of active substances. Carrier erythrocytes are prepared by collecting blood sample from the organism of interest and separating erythrocytes from plasma by centrifugation method. Through various physical and chemical methods the drug is loaded into the erythrocytes, finally they are resealed and the product carriers are then called as "resealed erythrocytes". Encapsulated drugs releases from this type of erythrocyte by phagocytosis or some other mechanisms. The application of this resealed erythrocyte is very effective as they are used as a targeted drug carrier of various anti cancerous drugs, anti viral drugs and various enzymes for enzyme therapy. There are also some recent developments is happening like erythrosome, nanoerythrosome those are the advanced version of resealed erythrocytes and more target specific in nature.

Keywords: Resealed Erythrocytes, Encapsulation, Phagocytosis

Introduction

Current view of pharmaceutical technology is aimed at development of drug delivery systems which maximize the drug targeting along with high therapeutic benefits with minimum side effects. Drug targeting can be approaches by either chemical modification or by appropriate carrier. 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. Leukocytes, platelets and erythrocytes have been proposed as cellular carrier systems. Erythrocytes are also biocompatible, biodegradable, possess very long circulation half-lives and can be loaded with different types of active components. Application of this resealed erythrocytes as promising slow drug release and site-targeted delivery systems for a variety of bioactive agents from different fields of therapy has gained.

Erythrocytes

Erythrocytes are highly specialized carrier for oxygen in the body which is transported via the circulatory system. Erythrocytes are the most abundant cells in the human body (~5.4 million cells/mm3 blood in a healthy male and ~4.8 million cells/mm3 in healthy female) and they are biconcave in nature. The flexible, biconcave shape enables erythrocytes to squeeze through narrow capillaries. They are rich in haemoglobin, an iron containing biomolecules that can transport oxygen from lungs to the all cells in the body. It is responsible for the blood red colour. The red blood cells develop in the bone marrow & circulate for about 100-120 days in the body until their components are recycled by macrophages. The processes of erythrocyte formation is called as erythropoiesis [1-2] [Figure 1].

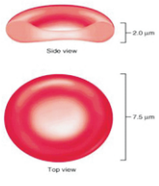

Figure 1: Side view and top view of RBC

Resealed erythrocytes

The carrier erythrocytes are prepared by collecting the blood samples from the organism of interest, then the erythrocytes are separating from the plasma, After this the drug of interest is injected into the erythrocyte by different methods. The drug loaded erythrocytes are known as resealed erythrocyte.Through the process of reinjection, the drug loaded erythrocytes provide slow circulating depots & target the drug to a disease tissue organ.

Advantages of resealed erythrocytes as a drug carrier [3-5]

·A remarkable degree of biocompatibility is the main advantage of this type of cellular carrier.

·They are completely biodegradability and produces minimum toxic product(s) resulting from the carrier biodegradation.

·They give considerable protection of the organism against the toxic effects of the encapsulated drug, e.g. antineoplasts.

·Their life-span is relatively longer in comparison to the synthetic carriers.

·Desirable size range and the considerably uniform size and shape.

·Possibility of targeted drug delivery to the RES organs.

·Relatively inert intracellular environment.

·Possibility of ideal zero-order kinetics of drug release.

·Wide variety of compounds with the capability of being entrapped within the erythrocytes.

·Modification of the pharmacokinetic and pharmacodynamic parameters of the drug.

Disadvantages [4-5]

·The major problem regarding this natural cells as drug carriers is that they are removed in-vivo by the RES as result of modification that occurred during drug loading procedure in cells.

·Another big but rare disadvantage of this type of drug carriers is the rapid leakage of certain encapsulated substances from the loaded erythrocytes.

·There are several molecules that may alter the physiology of the erythrocyte can't be loaded.

Isolation of erythrocyte [6-7]

·First blood is collected from the animal of interest into by venipuncture.

·Then the whole blood is centrifuged at 2500 rpm for 5 min. at 4 ±1 °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 °C until used.

·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. Fresh whole blood is generally used encapsulation. Because the efficiency of the erythrocytes isolated from fresh blood is much greater than the aged blood. After collecting the fresh whole blood it is immediately chilled to 4°C and stored for less than two days. The erythrocytes are then harvested and washed by centrifugation. The washed cells are suspended in buffer solutions at various hematocrit values as desired and are often stored in acid-citrate-dextrose buffer at 4°C for as long as 48 h before use.

Drug loading techniques inside the erythrocyte [7-10]

Endocytosis

This is a method of drug injection into the RBCs. The performance of this process depends on the drug concentration, pH (7.9–8.1), and temperature (37°C). However, the method is only suitable for the entrapment of cations or anions, which have both hydrophobic and hydrophilic groups [Figure 2].

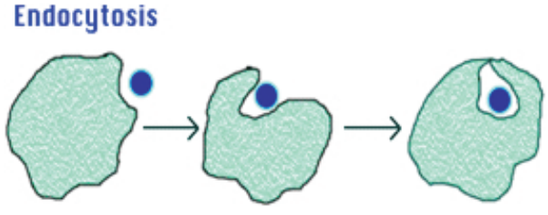

Figure 2 : Endocytosis

Electroporation

In this process electric shock is given to the erythrocytes that makes the erythrocyte membrane permeable for the drugs in isotonic solution. The potential difference created across the membrane leads to the formation of pores in erythrocyte membrane. Drugs can enter through these pores. This method has been successfully used for the encapsulation of several molecules and macromolecules in human erythrocytes but unfortunately this method is not suitable for large scale use.

Osmotic based method

Osmotic based methods are easy and flexible for handling large scale amount of blood. These methods are all based on the osmotic pressure difference between the inside and outside of the erythrocyte. Among the three existing methods (hypotonic preswelling, hypotonic dilution, and hypotonic dialysis), the hypotonic preswelling and the hypotonic dialysis processes remain the more suitable ones. This is one of the common method used in large scale production of resealed erythrocytes.

Hypotonic preswelling

This Method was developed by Rechsteiner in 1975 and was modified by Jenner et al. This is the technique which is based on the controlled swelling of erythrocytes. In this technique firstly the collected erythrocytes are washed with slightly hypotonic buffered solution. Under these conditions, the most fragile cells hemolyze and the remaining intact ones swell until reaching roughly 150% of their initial volume. After the centrifugation is done, the supernatant is discarded and the pellet of preswelled RBCs is isolated. In the second step, a hypotonic aqueous solution of the drug or API is prepared and carefully added to the washed erythrocytes in small volumes. Between each drug addition step, the suspension is centrifuged. The process is continued until a lysis point is reached (disappearance of distinct frontier between packed cells and the supernatant after centrifugation). RBCs are then resealed by adding a properamount of isotonic buffer and then incubation at 37°C. The operation takes roughly 2 hours [Figure 3, 4].

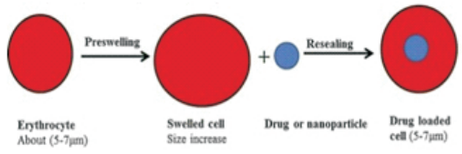

Figure 3 : Hypotonic preswelling

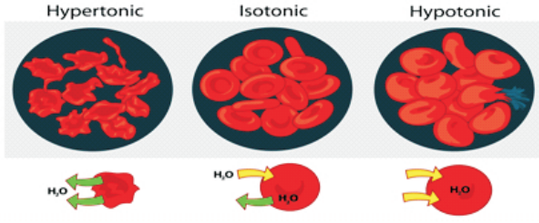

Figure 4 : Hypotonic Preswelling

Hypotonic dialysis

This method was first reported by Klibansky in 1959 and was used in 1977 by Deloach and Ihlerand Dale for loading of enzymes and lipids. This method is based on the principle that semipermeable dialysis membrane maximizes the intracellular: extracellular volume ratio for macromolecules during lysis and resealing. In the process, an isotonic, buffered suspension of RBCs with a specific hematocrit value in between 70-80 is prepared and placed in a conventional dialysis tube which is immersed in 10-20 volumes of a hypotonic buffer. The medium is agitated slowly. 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 [Figure 5].

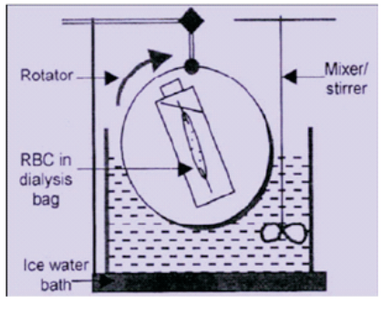

Figure 5: Hypotonic Dialysis technique

Hypotonic dilution

In this method, a certain amount of packed RBCs is diluted with 2-20 volumes of aqueous solution of a drug. The tonicity of the solution is then restored by adding a hypertonic buffer solution. After that the

resultant mixture is then centrifuged, the supernatant is discarded, and the pellet is washed with isotonic buffer solution. This process needs to reduce the circulation half life of the loaded RBCs.

Isotonic osmotic lysis

This method, also known as osmotic pulse method. It involves isotonic hemolysis that is achieved by physical or chemical means. If RBCs 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 heamolysis. The suspension was diluted with an isotonic-buffered drug solution. After the cells were separated, they were resealed at 37 °C.

Electro-insertion or Electro-Encapsulation

This procedure involves suspending RBCs in an isotonic buffer solution 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 1-10 kW/cm and optimal discharge time is between 20-160. The compound to be entrapped is added to the medium in which the cells are suspended from the commencement of the experiment [Figure 6].

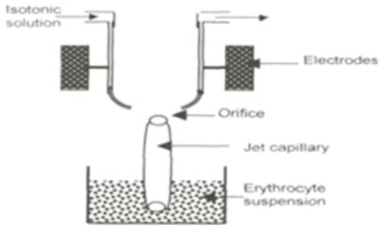

Figure 6: Electro encapsulation technique

Release mechanism of Loaded Drugs:

There are mainly three ways for a drug release from the erythrocyte carriers

Phagocytosis: By the process of phagocytosis normally erythrocyte cells removed from the blood circulation.

Diffusion through the membrane of the cells: Diffusion through the membrane depends on the drug molecule penetrate through a lipid bilayer i.e. bioactive compound have lipid solubility.

Using a specific transport system: Most of the drug molecules enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes.

***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 [11].

***In-vitro* characterization of Loaded Erythrocytes [12-14]**

Physical characterisation

Shape and surface morphology: The morphology of erythrocytes decides their life span after administration as a carrier. Light microscopy shows no observable changes in the resealed RBCs but in few cases spherical erythrocytes (spherocytes) are detected. SEM studies shows that a majority of the cells maintain their biconcave discoid shapes after the loading procedure.

Deformability: Shape change (deformability) is another factor that affects the life span of the RBCs. 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.

Drug content: This is also an important parameter for the measurement of effectiveness of the resealed RBCs as a drug carrier. Optimum drug content allows the encapsulation capacity of the resealed RBCs.

Cellular characterisation

Osmotic fragility: The osmotic fragility is the indicator of the possible changes in cell membrane integrity and the resistance of these cells to osmotic pressure of the suspension medium.

Turbulent fragility: It is another characteristic feature that depends upon changes in the integrity of cellular membrane and reflects resistance of loaded cells against hemolysis resulting from turbulent flow within circulation.

Biological characterisation

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

In vivo consideration

The efficacy of resealed erythrocytes is determined mainly by their survival time in the circulation system upon reinjection. The circulation survival kinetics of resealed erythrocytes show typical bimodal behavior with a rapid loss of cells during the first 24 h after injection, followed by a slow decline phase with a half life on the order of days or weeks.

Application of resealed erythrocytes

Slow drug release

Erythrocytes have been used as circulating depots for the sustained delivery of several types of drugs like antineoplastics, antiparasitics, veterinary antiamoebics, vitamins, steroids, antibiotics and cardiovascular drugs.

Drug targeting

Resealed erythrocytes can act as drug carriers and targeting tools as well. Surface-modified erythrocytes are used to target organs of mononuclear phagocytic system/ reticuloendothelial system because the changes in the membrane are recognized by macrophage.

Targeting RES organs

Damaged erythrocytes are rapidly cleared from circulation by Kupffer cells in liver and spleen in our body. Resealed erythrocytes loaded with desired active biomolecule, by modifying their membranes, can therefore be used to target the liver and spleen.

Treatment of hepatic tumors

Hepatic tumors are one of the most common types of cancer. Antineoplastic agents like methotrexate ,bleomycin, have been successfully delivered by resealed erythrocytes for the treatment of hepatic tumors.

Enzyme therapy

Enzymes can be administered into the patient's body with the help of resealed RBCs, to replace a missing or deficient enzyme in metabolic disorders like lysosomal storage disorders such as Gaucher's disease, hyperarginiaemia, hyperuricaemia and kidney failure.

Delivery of antiviral agents

Several reports show that different antiviral agents can be entrapped in resealed erythrocytes for effective delivery and targeting. As most

of the antiviral agents are nucleotides or nucleoside analogs, their entrapment and exit through the membrane needs careful consideration.

Recent Development

Erythrosome: Erythrosomes are one of the most specially designed vesicular carrier systems in which the cytoskeletons of human erythrocytes are used as sport upon which a lipid bilayer is coated. It is specially designed for macromolecular drugs. The results of the present study suggest that erythrosomes may be useful for membrane transport protein reconstitution and encapsulation systems.

Nanoerythrosomes(nEs): Nanoerythrosomes are also like erythrosomes prepared by the extrusion of RBC ghost. The drugs can be conjugated to the Nanoerythrosomes using certain cross linking agents like gluteraldehyde. They are characterized by various parameters like surface morphology, percent of drug conjugation, centrifugal stress, in vitro release etc.

Conclusion

Now a day's there are various applications have been proposed for the use of resealed erythrocytes as a drug carrier. Resealed erythrocytes show potential for a safe, effective and targeted delivery of various drugs. However, the concept of this encapsulation technique needs further optimization to be converted into a regular and routine drug delivery system.

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