

## AN OVERVIEW ON RESEALED ERYTHROCYTES: A NOVEL APPROACH TO DRUG DELIVERY

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## ABSTRACT

The number of products based on new drug delivery systems has significantly increased in the past few years and this growth is expected to continue in the near future. The reasons for this increasing interest in drug delivery are due to the increasing need of safe drugs, capable of reaching the target and with minimal side effects. In fact the main problems associate with systemic drug administration is essentially related to the bio-distribution of pharmaceuticals throughout the body. To overcome this problem and improving patient compliance as well as efficiency novel drug delivery through resealed erythrocytes is used now a days. Resealed Erythrocytes (RSE) are biocompatible, biodegradable, possess long circulation half-life 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 using various methods the cells are broken and the drug is entrapped into the erythrocytes, finally they are resealed and the resultant carriers are then called resealed erythrocytes.

**Keywords:** NDDS, Biodistribution, Biodegradable, RSE.

## INTRODUCTION

Current research is aimed at development of drug delivery system (DDS) with maximum therapeutic Benefits. Concepts are based on controlled drug delivery that is Biotechnology, polymer sciences and pharmaceutical sciences. 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. Target specificity would also dose effectiveness by reducing the dosage and frequency of administration. 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, Micro particles, cells, cell ghosts, lipoproteins, liposome's, erythrocytes). 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<sup>1</sup> Erythrocyte is red cell, biconcave discs, enucleate filled with hemoglobin (Hb), a protein that functions in gas transport. It contains the plasma protein spectrin, in healthy adult male=4. 5millions/ $\mu$  ml and in healthy adult female=4. 8million/ $\mu$  ml. Immature RBC are called Reticulocytes. <sup>2</sup>

## Composition of Erythrocytes

Blood contains 55% of plasma and other 45% made of corpuscles. Erythrocytes have diameter ranging from 6-9  $\mu$  and the thickness is nearly 1-2  $\mu$ . Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water. Lipidcontent of erythrocytes includes cholesterol, lecithin and cephalins. The concentration of K<sup>+</sup> is more in erythrocytes and Na<sup>+</sup> in plasma. The osmotic pressure of the interior of the erythrocytes is equal to that of the plasma and termed as isotonic (0. 9% NaCl or normal physiological saline. ) Changes in the osmotic pressure of the medium surrounding the red blood cells changes the morphology of the cells. If blood is placed into a tube and centrifuged, the cells and the plasma will separate. The erythrocytes, which are heavy, will settle down to the bottom of the tube, while the plasma rises up to the top and the leukocytes and platelets will form a thin layer (buffy coat) between the erythrocytes and the plasma. The haematocrit <sup>3</sup> is defined as the percentage of whole blood made up of erythrocytes.

Properties of resealed erythrocyte of novel drug delivery carriers<sup>4-8</sup>

1. The drug should be released at target site in a controlled manner.
2. It should be appropriate size, shape and should permit the passage through capillaries. and minimum leakage of drug should take place.
3. It should be biocompatible and should have minimum toxic effect.
4. It should possess the ability to carry a broad spectrum of drug.
5. It should possess specific physicochemical properties by which desired target size could be recognized.
6. 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.
7. The carrier system should have an appreciable stability during storage.

Advantage<sup>9-11</sup>

- 1) They are natural part of body, so they are biodegradable in nature.
- 2) The entrapment of drug does not require the chemical modification of drugs
- 3) The entrapment of drug also does not require the chemical modification of the substance to be entrapped.
- 4) They are non immunogenic in action and can be targeted to disease tissue/organ..
- 5) They prolong the systemic activity of drug.
- 6) Isolation of erythrocyte is easy and larger amount of drug can be encapsulated in small volume of cells
- 7) They can target the drug within reticuloendothelial system.
- 8) They facilitate incorporation of protein and nucleic acid in eukaryotic cells by cell infusion with RBC.

Limitations <sup>12</sup>

- 1) Limited potential as carrier to nonphagocytic target issue
- 2) Possibility of clumping of cells
- 3) Dose dumping may be also there.

**Requirement for encapsulation<sup>13-15</sup>**

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 HCl salt can be appreciably entrapped in bovine RBC. Generally, molecule should be polar. And Non polar molecule should 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 Bgalactosidase.

**Isolation of erythrocyte<sup>16</sup>**

The cellular content is about 40-50% of the blood volume and contains erythrocytes (red blood cells, RBC), Leukocytes (white blood cells, WBC) and thrombocytes (platelets). The primarily water (90 to 92%) and protein (7%). Blood is withdrawn from cardiac/splenic puncture (in case of small animal) and through veins (in case large animals) into a syringe containing drop of anticoagulant. The whole blood is centrifuged at 2500 rpm for 5min at 4± 4°C in a refrigerated centrifuge. The serum and Buffy coats are carefully removed and packed cells washed 3 times with phosphate buffer saline (PBS pH 7.4). The washed Erythrocyte are diluted with PBS and stored at 4°C until used.

**Table 1: Various condition and centrifugal force for isolation of erythrocytes<sup>17</sup>**

Sr.no.	Species	Washing Buffer	Centrifugal force(g)
1	Rabbit	10mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	500-1000
2	Dog	15mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	500-1000
3	Human	154mmol NaCl	<500
4	Mouse	10mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	100-500
5	Cow	10-15mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	1000
6	Horse	2mmol MgCl <sub>2</sub> , 10mmol glucose	1000
7	Sheep	10mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	500-1000
8	Pig	10mmol KH <sub>2</sub> PO <sub>4</sub> /NaHPO <sub>4</sub>	500-1000

**Entrapment Method****1. Hypo-osmotic lysis method**

- A) Hypotonic dilution or dilution method
- B) Preswell dilution method haemolysis
- C) Dialysis method
- D) Isotonic osmotic lysis method

**2. Chemical perturbation****3. Electro insertion method****4. Endocytosis method****5. Normal transport method****6. Lipid fusion method****Entrapment Method****(1) Hypo-osmotic lysis method<sup>18-19</sup>**

Hypotonic lysis of cells in a solution containing the drug/enzyme to be entrapped followed by restoration of tonicity in resealed them. The ghost population obtained are heterogeneous and they are three types. The type I ghosts which resealed immediately after haemolysis, type II ghosts which resealed after reversal of haemolysis by addition of alkali ions and type III ghosts which remain leaky under different experimental conditions. Erythrocyte 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 biconcave, discocyte (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 ruptures, permitting escape of the cellular component. Erythrocytes are resealed on addition of sufficient 1.54 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<sup>20-22</sup>**

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.

By using a new apparatus, it is possible to entrap a variety of biological compound into erythrocytes in as little time as 2 hours at room temperature under blood banking condition; the method is based on two sequential and controlled hypotonic dilutions of washed red blood cells followed by concentration with a haemofilter. Subsequent isotonic resealing of erythrocytes allow a 35-50% cell recovery and approximate 30% of added drug

**A) Hypotonic dilution or Dilutional method<sup>23-24</sup>**

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 a 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 Bgalactosidase and B-glucosidase, asparaginase, and arginase, as well as bronchodilators such as salbutamol.

**(B) Preswell dilutional haemolysis<sup>25-26</sup>**

This Method was developed by Rechsteiner in 1975 and was modified by Jenner et al. for drug loading. The technique is based upon initial controlled swelling of erythrocytes in a hypotonic buffered solution without lysis. 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 ml portions of an aqueous solution of the drug to be encapsulated. 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. Then, the cell suspension is incubated at 37°C to resealed the resealed erythrocytes. This method is simpler and faster than other methods, causing minimum damage to cells. Drugs encapsulated in erythrocytes using this method include propranolol, asparaginase, cyclophosphamide, cortisol-21-phosphate, w1-antitrypsin, methotrexate, insulin, metronidazole, levothyroxine, enalaprilate and isoniazide.

**(C) Hypotonic dialysis<sup>27-28</sup>**

This method was first reported by Klibansky Dale for loading enzymes and lipids. In the process, an isotonic, buffered suspension of erythrocytes with a haematocrit value of 70-80 is prepared and placed in a conventional dialysis tube immersed in 10-20

volumes of a hypotonic buffer. The medium is agitated slowly for 2 hr. 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. 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. In this method, the erythrocyte suspension and the drug to be loaded was placed in the blood compartment and the hypotonic buffer was placed in a receptor compartment. This led to the concept of "continuous flow dialysis. 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. This method has been used for loading enzymes such as B - galactosidase, glucoserebrosidase, asparaginase, inositol, hexaphosphatase as well as drugs such as gentamicin, adriamycin, pentamidine and furamycin, interleukin-2 and human recombinant erythropoietin.

#### (D) Isotonic osmotic lysis<sup>29</sup>

This method is known as the osmotic pulse method, involves isotonic hemolysis that is achieved by physical or chemical means. The isotonic solutions may or may not be isotonic. 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. However, this method also is not immune to changes in membrane structure composition.

#### (2) Chemical perturbation of the membrane<sup>30, 31</sup>

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 used halothane for the same purpose. However, these methods induce irreversible destructive changes in the cell membrane and hence are not very popular.

#### 3) Electro-insertion or electroencapsulation<sup>32, 33</sup>

This method is also known as electroporation, the method consist of reating electrically induced permeability changes at high membrane potential differences. In 1977, Tsong and Kinosita suggested the use of transient electrolysis to generate desirable membrane permeability for drug loading. Electrical breakdown is achieved by membrane polarization for microseconds using varied voltage of 2kv/cm is applied for 20 µsec. The potential difference across the membrane is built up either directly by inter and intracellular electrodes or indirectly by applying internal electric field to the cells. The extent of pore formation depends upon the electric field strength, pulse duration and ionic strength of suspending medium. Once membrane is perforated, regardless of the size of pores, ions rapidly distribute between the extra and intracellular space to attain Donnan equilibrium, however the membrane still remains impermeable to its cytoplasmic macromolecules. In red blood cells, the colloidal osmotic pressure of haemoglobin is about 30 mOsm. This pressure drives water and ion influx, as a result swelling of the cells occurs. The membrane is ruptured when the cell volume reaches 155% of its original volume. Since the cell lysis is due to colloidal osmotic swelling, the rational to prevent lysis is to balance the colloidal osmotic pressure of cellular macromolecules. This can be affected by addition of large molecules (like tetrasaccharide stachyose or protein such as bovine serum albumin) and ribonucleases. This helps to counteract the colloidal osmotic swelling of electrically perforated erythrocytes. Under this osmotically balanced condition pores stay open at 4°C for few days. If drug molecules are added at this point, they permeate into red

blood cells. The various candidates entrapped by this method include primaquine and related 8-amino-quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine and vitamin A.

#### 4) Entrapment by endocytosis<sup>34</sup>

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<sub>2</sub>, and 1mM CaCl<sub>2</sub>, followed by incubation for 2 min at room temperature this method was reported by Schrier *et al.* in 1975. The pores created by this method are resealed by using 1.54 mM of NaCl and incubation at 37 °C 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. The various candidates entrapped by this method include primaquine and related 8-amino-quinolines, vinblastine, chlorpromazine and related phenothiazines, hydrocortisone, propranolol, tetracaine, and vitamin A

#### 5) Normal transport system<sup>35</sup>

It is possible to load erythrocytes with drug without disrupting the erythrocyte membrane in any way by incubating the drug and erythrocytes for varying period of time. After infusion the drug would in general exit from the cell following the kinetics comparable to those observed for entry.

#### 6) Lipid fusion technique<sup>36, 37</sup>

In this method fused lipid vesicle containing bioactive molecule along with human erythrocytes leading to exchange of lipid entrapped drug molecule. This method provides very low encapsulation efficiency. Nicolau and Gresonde fused lipid vesicle containing inositol hexaphosphate with human erythrocytes. The incorporated inositol hexaphosphate in erythrocytes provided a significant lowering of the oxygen affinity for haemoglobin in intact erythrocytes. Harrison et al reported resealing of tyrosine kinase into human erythrocytes by rapid freezing and thawing in liquid.

#### Characterization of Resealed Erythrocytes<sup>38-40</sup>

##### Drug content quantification

To determine the drug content, packed loaded cells are deproteinized with acetonitrile after centrifugation at 3000 rpm for a fixed time interval. The clear supernatant liquid is analysed spectrophotometrically.

##### In-vitro drug release and haemoglobin content study

In-vitro release of drug(s) and haemoglobin are monitored periodically from drug loaded cells. The cells suspension (5% hematocrit in PBS) is stored at 4°C in amber coloured glass containers. Periodically the clear supernatant are withdrawn using a hypodermic syringes equipped with 0.45 µm filter, deproteinized using methanol and were estimated for drug content.

The supernatant of each sample after centrifugation is collected and assayed, % haemoglobin release may be calculated using the formula.

$$\% \text{ haemoglobin release} = \frac{A_{540} \text{ of sample} - A_{540} \text{ of background}}{A_{540} \text{ of 100\% haemoglobin}}$$

Or

$$\text{Mean corpuscular hemoglobin (MCH (pg))} = \frac{\text{Haemoglobin (g/100ml)} \times 10}{\text{Erythrocyte count (millions/cu mm)}}$$

Erythrocyte count (millions/cu mm)

Where a A540 refers to absorbance at 540nm.

##### Percent cell recovery and Morphological study

Percent cell recovery may be determined by counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug. Phase contrast or electron microscope may be used for normal and drug loaded erythrocytes.

### Osmotic fragility and Osmotic shock study

To study the effect of different tonicities, drug loaded erythrocytes are incubated separately in normal saline solution at  $37 \pm 2^\circ\text{C}$  for 10 minutes, followed by centrifugation at 2000 rpm for 10 min. For osmotic shock study, dispersing the resealed erythrocyte suspension in distilled water and centrifuged at 300 rpm for 15 min. The supernatant was estimated for percent haemoglobin release spectrophotometrically.

### Turbulence shock study

It is the measure of simulating destruction of loaded cells during injection. Normal and drug loaded cells are passed through a 23 gauge hypodermic needle at a flow rate of 10 ml/min which is comparable to the flow rate of blood. It is followed by collection of an aliquot and centrifugation at 2000 rpm for 10 minutes. The haemoglobin in withdrawn sample is estimated.

Drug loaded erythrocytes appear to be less resistant to turbulence, probably indicating destruction of cells upon shaking.

### Erythrocyte sedimentation rate (ESR)

It is an estimate of the suspension stability of RBC in plasma and is related to the number and size of the red cells and to relative concentration of plasma protein, especially fibrinogen and  $\alpha$ ,  $\beta$  globulins.

This test is performed by determining the rate of sedimentation of blood cells in a standard tube. normal blood. ESR is 0 to 15 mm/hr. higher rate is indication of active but obscure disease processes.

### Entrapped magnetite study

The hydrochloric acid is added to a fixed amount of magnetite bearing erythrocytes and contents are heated at  $60^\circ\text{C}$  for 2 hr. Then 20% w/v trichloroacetic acid is added and supernatant obtained after centrifugation is used to determine magnetite concentration using atomic absorption spectroscopy.

### Self life and Stability and Cross linking of Released Erythrocytes

Glutaraldehyde (0. 2%) treated erythrocytes in a sintered glass funnel (G-4) by filtration and dried in vacuum (200mm Hg) for 10 hr. alternatively the erythrocyte suspension was filled into vials and lyophilized at  $-40^\circ\text{C}$  to 0. 01 torr using a laboratory lyophilizer. The dried powder was filled in amber colour glass vials and stored at  $4^\circ\text{C}$  for month. Improvement in shelf life of the carrier erythrocytes was achieved by storing the cells in powder form, ready for reconstitution at  $4^\circ\text{C}$ .

### Storage

Stored encapsulated preparation without loss of integrity when suspended in hank's balanced salt solution (HBSS) at  $4^\circ\text{C}$  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.

### Mechanism of Drug Release from Resealed Erythrocyte

There are mainly three ways for a drug to efflux out from erythrocyte carriers.

1. Phagocytosis
2. Diffusion through the membrane of the cell and
3. Using a specific transport system.

RBCs are normally removed from circulation by the process of phagocytosis. The degree of cross-linking determines whether liver or spleen will preferentially remove the cells Carrier erythrocytes following heat treatment or antibody cross-linking are quickly removed from the circulation by phagocytic cells located mainly in liver and spleen. The rate of diffusion depends upon the rate at which a particular molecule penetrates through a lipid bilayer. It is greatest for a molecule with high lipid solubility. Many substances enter cells by a specific membrane protein system because the carriers are proteins with many properties analogous to that of enzymes, including specificity.

### Route of administration<sup>41</sup>

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.

### Application<sup>42-44</sup>

#### (1) In-Vitro Application :

For in vitro phagocytosis cells have been used to facilitate the uptake of enzymes by phagolysosomes. Enzymes content within carrier RBC could be visualized with the help of cytochemical technique. The biochemical defects such as the glucose- 6-phosphate dehydrogenase (G6PD) deficiency can be useful tool for discerning the mechanism that eventually causes these effects.

The most frequent in vitro application of RBC is that of micro-injection. A protein or nucleic acid was 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 has been used to confirm the site of action of fragment of diphtheria toxin. Antibodies introduced using RBC mediated microinjection is recorded not to enter the nucleus, thus limiting the studies to the cytoplasmic level.

#### (2) In - Vivo Application

##### i) Targeting of bioactive agents to RE System

Damaged erythrocytes are rapidly cleared from circulation by phagocytic Kupffer cells in liver and spleen. Resealed erythrocytes, by modifying their membranes, can therefore be used to target the liver and spleen. The various approaches to modify the surface characteristics of erythrocytes include surface modification with antibodies, glutaraldehyde, carbohydrates such as sialic acid and sulphhydryl.

##### ii) Targeting to sites as other than RES Organs

Resealed erythrocytes have the ability to deliver a drug or enzyme to the macrophage-rich organs. Organ targeting other than RES have been tried recently with resealed erythrocytes. Some of the representative approaches are discussed in brief.

##### iii) Erythrocytes as Circulating Bioreactors

Erythrocytes have been realized as carriers for enzymes to serve as circulating bioreactors. Sometimes it is desirable to decrease the level of circulating metabolites that can enter erythrocytes. Erythrocytes have also been used as circulating bioreactors for the controlled delivery of antiviral drugs.

##### iv) Erythrocytes as Carriers for Drugs

Various bioactive agents encapsulated in erythrocytes are developed for the slow and sustained release in circulation to allow effective treatment of parasitic disease. Resealed erythrocytes serve as an ideal carrier for antineoplastic agents, antimicrobial drug and vitamins and steroids.

##### v) Erythrocytes as Carriers for Enzymes

Enzymes can be injected into the blood stream to replace a missing or deficient enzyme in metabolic disorders or to degrade toxic compounds accumulated in the blood due to a disease likewise, environmental, lysosomal storage disorders such as Gaucher's disease, hyperargininaemia, hyperuricaemia, hyperphenyl- alaninaemia and kidney failure are only few examples of metabolic disorders that can be treated by administration of enzymes.

## Recent developments

### Nanoerythroosomes

Nanoerythroosomes are vesicles prepared by the extrusion of RBC ghosts, the average diameter of these vesicles being 100nm. The process gave small vesicles with the size of a liposomes. These spheroid particles were named 'nanoerythroosomes' and appear to be stable and maintain both the cytotoxic and antineoplastic activity of daunorubicin against mice leukaemia P338D cells. Significant advances have been made with the use of erythrocyte for specific targeting to cells of the immune system antiviral drugs can be pretreated to deliver drug directly to macrophages.

Several laboratory techniques have developed for the encapsulation of allosteric effector of haemoglobin, inositol hexaphosphate, which are effective at oxygen delivery, much more effective than normal erythrocytes.

### CONCLUSION

The resealed technology has given an active arena for the further research. The commercial medical applications of carrier erythrocytes are currently being tested in Europe by a recently formed company that is developing products for human use. The coming years represent a critical time in this field as commercial applications are explored. In near future, erythrocytes based delivery system with their ability to provide controlled and site specific drug delivery will revolutionize disease management.

The International Society for the use of Resealed Erythrocytes (ISURE) through its biannual meetings provides an excellent forum for exchange of information to the scientist in this exciting and rewarding field of research. For the present, it is concluded that erythrocyte carriers are most effective in novel drug delivery systems considering their tremendous potential. The future studies would concentrate on manipulation of the autologous properties of erythrocytes, improved understanding biology of the red cells and its membrane, development of pulsatile and feedback control system, selective drug delivery to CNS and delivery of peptide and protein drugs. Main suggestion for future study is that by carrier through we can transplant steroids and hormones to the targeting site. So we can decrease many side effect. In these field no one can deep think but by resealed erythrocyte we can improvise drug targeting area and reduces so many side effect.

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