INTRODUCTION

• Amongst various carriers explored for target oriented drug delivery, vesicular, micro particulate & cellular carriers meet several criteria rendering them useful in clinical applications.

• Erythrocytes have been the most extensively investigated and found to posses great potential in novel drug delivery.

• Erythrocytes are loaded with drug/enzymes & provide target drug delivery system.

• 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. Hence, these carriers are called resealed erythrocytes.
Erythrocytes

- Erythro = red
- Cyt = cell

- Biconcave discs, anucleate.

- Filled with hemoglobin (Hb), a protein that functions in gas transport

- **Erythrocyte ghosts**: RBC without hemoglobin
DRUG CARRYING POTENTIAL OF RBC

- The developing RBC has capacity to synthesize hemoglobin, however, adult RBCs do not have this capacity and serve as carriers for hemoglobin.

- The carrier potentials of these cells was first realized in early 1970.

- Drug which are normally unable to penetrate the membrane, should be made to transverse the membrane without causing any irreversible changes in the membrane structure and permeability.
• Cells must be able to release the entrapped drug in a controlled manner upon reaching the desired target.

• The processing of drug entrapment requires a reversible and transient permeability change in the membrane, which can be achieved by various physical and chemical means.
Why Resealed Erythrocytes??

- Biodegradability with no generation of toxic products
- Wide range of chemicals can be entrapped
- Ease of circulation
- Biocompatibility probably no changes of triggered immune response
- Ability to target RES organs
Limitations

- They have a limited potential as carrier to non-phagocytic target tissue.

- Possibility of Leakage of the cells and dose dumping may be there.
Source and isolation of RBC

- 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.

- To isolate erythrocytes, blood is collected in heparinized tubes by venipuncture.

- Fresh whole blood is typically used for loading purposes because the encapsulation efficiency of the erythrocytes isolated from fresh blood is higher than that of the aged blood.

- Fresh whole blood is the blood that is collected and immediately chilled to 4° C and stored for less than two days.
Effects of tonicity on RBCs

- Isotonic solution
- Hypotonic solution: \( H_2O \)
- Hypertonic solution: \( H_2O \)
- Crenated
Drug Loading in Resealed Erythrocytes

- Membrane Perturbation
  - Dilution method
- Electro encapsulation
  - Dialysis method
- Hypo-Osmotic Lysis
  - Preswell method
- Lipid fusion, Endocytosis
  - Osmotic lysis
Dilutional Haemolysis

RBC $\xrightarrow{0.4\% \text{ NaCl}}$ Membrane ruptured RBC $\xrightarrow{\text{Drug}}$ Loaded RBC

Hypotonic Loading buffer Incubation at 25ºC Resealing buffer

Efficiency $\rightarrow$ 1-8% Enzymes delivery

Washed Resealed Loaded RBC
Isotonic Osmotic Lysis

Physical rupturing

RBC

Chemical rupturing

Isotonically ruptured RBC
Drug
Loaded RBC
Incubation at 25°C
Resealed RBC

Chemical – urea, polyethylene, polypropylene, and NH₄Cl
Preswell Dilutional Haemolysis

RBC → 0.6% w/v NaCl → Swelled RBC

5 min incubation at 0 °C → Drug + Loading buffer → Loaded RBC

Incubation at 25 °C → Resealing Buffer → Resealed RBC

Material to be entrapped

Hypotonic medium

Efficiency → 72%

Fig:- Preswell Method
Dialysis

80% Haematocrit value

RBC + Phosphate buffer → Placed in dialysis bag with air bubble → Dialysis bag placed in 200ml of lysis buffer with mechanical rotator 2hrs. 4c. → Loading buffer

Drug

Loading buffer

Dialysis bag placed in Resealing buffer with mechanical rotator 30 min 37c. → Loaded RBC

Efficiency → 30-45%
Electro-insertion or Electro-encapsulation

RBC + 2.2 Kv Current for 20 micro sec
Pulsation medium
At 25°C
Loading suspension
Drug
3.7 Kv Current for 20 micro sec
Isotonic NaCl
Loaded RBC
Resealing Buffer
Resealed RBC

Isotonic solution
Electrodes
Orifice
Jet capillary
Erythrocyte suspension

Fig:- Electro-encapsulation Method
Entrapment By Endocytosis

RBC + Drug + Buffer containing ATP, MgCl₂, and CaCl₂ At 25°C → Loaded RBC → Resealed RBC

Drug Loaded Erythrocyte

Fig:- Entrapment By Endocytos Method
Membrane perturbation method

RBC → Amphotericin B (e.g. Chemical agents) → Increased permeability of RBC → Drug (Resealing Buffer) → Resealed RBC
<table>
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<tr>
<th>METHOD</th>
<th>%LOADING</th>
<th>ADVANTAGES</th>
<th>DISADVANTAGES</th>
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<tbody>
<tr>
<td>Dilution method</td>
<td>1-8%</td>
<td>Fastest &amp; simplest especially for low molecular weight drugs</td>
<td>Entrapment efficiency is very less (1-8%)</td>
</tr>
<tr>
<td>Dialysis</td>
<td>30-45%</td>
<td>Better in vitro survival of membrane due to lesser ionic load</td>
<td>Time consuming; heterogeneous size distribution of resealed erythrocytes</td>
</tr>
<tr>
<td>Preswell dilution</td>
<td>20-70%</td>
<td>Good retention of cytoplasm constituents &amp; good survival in vivo.</td>
<td>-</td>
</tr>
<tr>
<td>Isotonic osmotic lysis</td>
<td>-</td>
<td>Better in vivo surveillance</td>
<td>Impermeable to large molecules, process is time consuming</td>
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IN VITRO CHARACTERIZATION

• Drug Content
Packed loaded erythrocytes (0.5 ml) are first deproteinized with acetonitrile (2.0 ml) and subjected to centrifugation at 2500 rpm for 10 min. The clear supernatant is analyzed for the drug content.

• In vitro Drug and Haemoglobin Release
Normal and loaded erythrocytes are incubated at 37± 2°C in phosphate buffer saline (pH 7.4) at 50% haematocrit in a metabolic rotating wheel incubator bath. Periodically, the samples are withdrawn with the help of a hypodermic syringe fitted with a 0.8μ Spectropore membrane filter. Percent haemoglobin can similarly be calculated at various time intervals at 540 nm spectrophotometrically.
• **Osmotic Fragility**
  When red blood cells are exposed to solutions of varying tonicities their shape changes (swell in hypotonic and shrink in hypertonic environments) due to osmotic imbalance. Assayed for Hb and/or drug release.

• **Osmotic Shock**
  Osmotic shock describes a sudden exposure of drug loaded erythrocytes to an environment, which is far from isotonic to evaluate the ability of resealed erythrocytes to withstand the stress and maintain their integrity as well as appearance.
• **Turbulence Shock**
  The parameter indicates the effects of shear force and pressure by which resealed erythrocytes formulations are injected, on the integrity of the loaded cells.

• Loaded erythrocytes (10% haematocrit, 5 ml) are passed through a 23-gauge hypodermic needle at a flow rate of 10 ml/min. After every pass, aliquote of the suspension is withdrawn and centrifuged at 300 G for 15 min, and haemoglobin content, leached out are estimated spectrophotometrically.

• **Morphology and Percent Cellular Recovery**
  Phase-contrast optical microscopy, transmission electron microscopy and scanning electron microscopy are the microscopic methods used to evaluate the shape, size and the surface features of the loaded erythrocytes.
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<td>TEM, SEM, Phase contrast</td>
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<td>optical microscopy</td>
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<tr>
<td>Vesicle size &amp; size distribution</td>
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<td>TEM, Optical microscopy</td>
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<td>Drug release</td>
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<td>Diffusion cell/ Dialysis</td>
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<td>% Encapsulation</td>
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<td>Deproteinization</td>
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<tr>
<td>Electrical surface potential &amp; pH</td>
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<td>Zeta potential and pH sensitive probes</td>
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<tr>
<th><strong>Cell related characterization</strong></th>
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<tr>
<td>% Hb content/volume</td>
<td></td>
<td>Deproteinization</td>
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<tr>
<td>Mean corpuscular Hb</td>
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<td>Laser light scattering</td>
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<td>Osmotic fragility</td>
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<td>Incubation with isotonic to</td>
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<td>hypotonic saline and estimation</td>
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<td>Osmotic shock</td>
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<td>Dilution with distilled water and</td>
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<td>Turbulent shock</td>
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<td>and estimation of drug/Hb</td>
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Erythrocyte Sedimentation Rate - ESR apparatus

✓ Biological Characterization

Sterility -- Aerobic or anaerobic cultures
Pyrogenecity -- LAL test
Animal toxicity --- Toxicity tests.
Shelf and Storage Stability of Resealed RBC

- 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.

- Exposure of resealed erythrocytes to membrane stabilizing agents such as dimethyl sulfoxide, dimethyl, 3,3-di-thio-bispropionamide, gluteraldehyde, toluene-2-4-diisocyanate followed by lyophilization or sintered glass filtration has been reported to enhance their stability upon storage.
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.
Applications of resealed erythrocytes

- Erythrocytes as carrier for enzymes
- Erythrocytes as carrier for drugs
- Erythrocytes for drug targeting
  - Drug targeting to reticuloendothelial system
  - Drug targeting to liver
    - Treatment of liver tumors
    - Treatment of parasitic diseases
    - Removal of RES iron overload
    - Removal of toxic agents
Drug Targeting to Liver

- Enzyme Deficiency/Replacement Therapy: Gaucher’s disease (glucocerebrosidase), replacement of enzyme in lysosomes (glucuronidase, galactosidase, glucosidase)

- Treatment of Liver Tumours

- Treatment of Parasitic diseases

- Removal of Toxic Agents: enzyme to hydrolyze organophosphorous compounds.
Drug Targeting to RES Organs

The damaged erythrocytes are quickly removed from circulation by phagocytic Kupffer cells located in liver and spleen.

Chemically modified RBC can be targeted to organs of the MPS.

- Surface Modification with Antibodies
- Surface Modification with Glutaraldehyde
- Surface Modification-involving Carbohydrates
- Surface Modification with Sulphhydryls
- Surface chemical cross-linking
Erythrocytes as circulating bioreactors

- Delivery of Antiviral Agents
- Delivery of Azidothymidine Derivative
- Delivery of Deoxycytidine Derivatives
- Macrophage Activation
- Thrombolytic Therapy
- Oxygen Deficiency Therapy
- Delivery of Interleukins
## Various Applications of Resealed Erythrocytes

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<th>APPLICATION</th>
<th>DRUG/ENZYME/ macromolecules</th>
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<td>Enzyme deficiency, &amp; Enzyme replacement Therapy</td>
<td>B-galactosidase, B-fructofuranodase, Urease, Glucose-6-phosphate dehydrogenase, corticol-2-phosphate</td>
</tr>
<tr>
<td>Thrombolytic activity</td>
<td>Brinase, Aspirin, Heparin</td>
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<tr>
<td>Iron overload chemotherapy</td>
<td>Desferroxamine, Rubomycin, Methotrexate, L-asparginase, Doxorubicin, Daunomycin, Cytosine, Arabinoside</td>
</tr>
<tr>
<td>Immuno therapy</td>
<td>Human recombinant interleukin-2</td>
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<tr>
<td>Circulating carriers</td>
<td>Albumin, Prednisolone, Salbutamol, Tyrosine kinase, Phosphotriesterase.</td>
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<tr>
<td>Circulating Bioreactors</td>
<td>Arginase, Uricase, Luciferase, Acetaldehyde dehydrogenase.</td>
</tr>
<tr>
<td>Targeting to RES</td>
<td>Pentamidine, Mycotoxin, Imidocarb Diproponate.</td>
</tr>
<tr>
<td>Targeting to other than RES</td>
<td>Daunomycin, Methotrexate, Diclofenac sodium.</td>
</tr>
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</table>
Nanoerythrosomes

- Extrusion of RBC ghosts to produce small vesicles having an average diameter of 100nm.

Erythrosomes

- Specially engineered vesicular systems in which chemically cross linked human erythrocyte cytoskeletons are used as a support upon which a lipid bilayer is coated.
REFERENCES

THANKS TO ONE & ALL