Resealed erythrocytes :-

Erythrocytes are biocompatible provided that compatible cells are used in patients, there is no possibility of triggered immunological response.

⇒ Resealed erythrocytes concepts starts in early 1970. Ihler et al. suggested that resealed erythrocytes could be useful as drug carrier.

* The term carrier RBC was first introduced in 1979.

Novel Drug carrier.

1. It should be Appropriate size, shape to permit the passage through the capillaries.
2. posses specific physico-chemical properties for target site
3. " Biocompatible and should have minimum toxic effect.
4. minimum leaching / leakage of drug before targetting.
5. Drug should be released at the target site in a controlled manner.
6. should have ability to carry broad spectrum of drugs with different properties.
7. physico-chemical compatible with the drug.
8. should have appreciable stability during storage.

Advantages and Limitations :- (Resealed erythrocytes).

1) They are natural product of body, biodegradable in nature.

2) Isolation of erythrocytes is easy and larger amount of drug can be encapsulated in a small volume.
3) Entrapment of drug doesnot require the chemical modification of the substances. (Avoid co-valent coupling with carrier → affect bioavailability).
4) They are non-immunogenic in ather and can be targeted to disease tissue/organ
5) They prolong the systemic activity of drug while residing for a longer time in body.
6) They protect the premature degradation, inactivation and excretion of proteins and enzymes and acts as a carrier for no. of drugs.
7) They can target the drugs within reticuloendothelial system (RES).

Limitations

1) They have a limited potential as carrier to non-phagocytic target tissue
2) possibility of clumping of cells and dose dumping may be there.
3)

Erythrocytes :-

Flexible, elastic, biconcave, nucleated structure with a mean dia. of 7.3 μm and thickness 2.2 μm.

Chemical composition water 63 %
lipid 0.5 %
glucose 0.8 %

minerals 0.7-1.
non hemoglobin protein - 0.9-1.
methehemoglobin 0.5-1.
hemoglobin 33.67 1.

Red cell membrane :- Dynamic, semi permeable component absorbed in energy metabolism in the maintenace of energy metabolism (Na^+, K^+, Cl^-, HCO_3^-)

* The stroma of insoluble material which remains after rbc cell disruption (hemolysis) constitutes 2 to 5-1. of the wet cell vol.
It is primarily protein (46 to 80 1.) and lipid 10-12 1.

Requirements after encapsulation :-

1) Wide range of biologically active substance (5000 — 600,000 daltons in size) can be entrapped
2) molecules should be polar or hydrophilic.
3) Once encapsulated, charged molecules are retained longer than uncharged molecules
4) Hydrophobic molecules can be entrapped in erythrocytes by absorbing over other molecules.
Ex Adriamycin actively interacts with the RBC membrane and destroy the cells. (so it is first bind with DNA segment then entrapped in RBC)

Non-polar molecules may be entrapped in erythrocytes in their respective salt. (Tetracycline HCl salt can be entrapped in Bovine RBC)

A man without a smiling face must not open a shop.

6) Isolation of erythrocytes :-

Cellular content is about 45-1. of the blood volume and contains erythrocytes (RBC), leucocytes (WBC) and thrombocytes.

Blood is withdrawn through veins into syringe
+
1 drop of anticoagulant (Previously in syringe)
↓
Centrifuged at 2500 rpm for 5 min at 4±1°C in a refrigerated centrifuge.
↓
Remove serum and buffy coats.
↓
Packed cells are washed 3 times with phosphate buffer saline (PBS, PH 7.4).
↓
Washed RBC are diluted with PBS and stored at 4°C.

Composition of Anticoagulants :- (per 10ml blood)

Lithium, potassium or sod. oxalate (15 to 25 mg)
Sodium citrate (40 to 60 mg)
heparin-sod (2 mg).
disod. EDTA 10 to 30 mg.

Friendship increases in visiting friends, but in visiting them seldom.

28 JUNE SATURDAY 2.0.0.3

JUNE

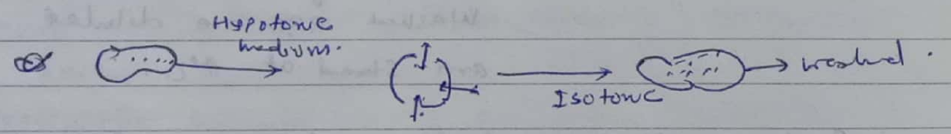
MO	TU	WE	TH	FR	SA	SU	WK
30						1	22
2	3	4	5	6	7	8	23
9	10	11	12	13	14	15	24
16	17	18	19	20	21	22	25
23	24	25	26	27	28	29	26

7) Entrapment methods :-

1 Hypo-osmotic lysis method.
   a) Dilution method
   b) Dialysis method.
   c) pressure method.
   d) Isotonic osmotic lysis method.
2. Electrical Breakdown method.
3) Endocytosis method.
4) Membrane perturbation method.
5) Normal transport method.
6) Lipid fusion method.

1) Hypo-osmotic lysis method :-

In this process the Intracellular and extracellular solutes of erythrocytes are exchanged by osmotic lysis. and resealing.



a) Dilution method :-

29 SUNDAY * The erythrocytes have little capacity to resist volume.
* At an ↑ in vol. above 50.7% of the initial vol and in hypotonic soln (C ≤ 0.4-0.45 M Cl) the erythrocyte membrane ruptures, escape of cellular content and equilibrium is achieved within one minute.

A wise enemy is better than a foolish friend.

JULY

MO	TU	WE	TH	FR	SA	SU	WK
	1	2	3	4	5	6	27
7	8	9	10	11	12	13	28
14	15	16	17	18	19	20	29
21	22	23	24	25	26	27	30
28	29	30	31				31

2.0.0.3 MONDAY JUNE 30

* Results into cell swelling upto 1.6 times its original vol.

* Due to swelling appearance of pores of 200-500 A° in size which allow equilibration of the intracellular and extracellular vol.

At 0°C pores open for longer period

* Then ↑ing the ~~Ionic~~. Ionic strength at 37°C results into resealing of cell membrane and restoring the osmotic properties of the erythrocytes.

Ex entrapment of β- glucosidase, β galactosidase.

Limitation :- * By this method encapsulation efficiency is 1-8%.
* Simplest method
* Efficient for encapsulation of low m.w. drugs.

b) Dialysis method :-

Desired hematocrit is achieved by mixing erythrocyte susp + Drug solution.

↓

This mixture is placed in dialysis unit and both end of tube are tied with thread.

↓

Air bubble of 25% of internal volume is left in tube

↓

Blood is thicker than water.

Tube is placed in a bottle contains 10ml of swelling solution.

↓

Tube is placed at 4°C for the desired dialysis time. (Stirred)

↓

Dialysis tube is placed in 100ml of resealing solution (Isotonic PBS, PH 7.4) at room temp 25-30°C for resealing

↓

Loaded erythrocytes washed 2 cold PBS at 4°C.

↓

Cells are resuspended in PBS.

Adv:- Good entrapment is obtained.

Ex ⇒ encapsulation of protein
⇒ In-vivo survival of RBC
⇒ entrapment of hexokinase, acetyladehyde dehydrogenase, bilirubin oxidase.

c) Preswell dilution method:-

① First ~~setti~~ swelling of erythrocytes without lysis by placing them in slightly hypotonic solution

↓

Recovered the swollen cell (centrifuge at low speed)

↓

Add relatively small aq. drug soln are added to point of lysis.

Happiness is a wondrous commodity; the more you give, the more you have.

Adv:- The slow swelling of cells reverts in good retention of cytoplasmic constituents and hence good survival in invivo.

20-28% drugs can be loaded.

72% reported in thyroxin encapsulated RBC.

d) Isotonic osmotic lysis technique:-

① Transient permeability in erythrocyte wall could be produced using propylene glycol which allows the drugs / agents to diffuse in.

② The lysed cell are resealed under Isotonic soln.

2) Electrical Breakdown:-

Opening of erythrocyte membrane based on an electrically induced permeability changes at a higher membrane potential difference.
(voltage of about 1 volt).

↓

At this voltage ↑ in membrane conductance is observed (by applying Internal electric field pulse to cell suspension).

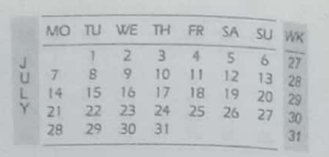
↓

Breakdown leads to formation of pores
(Breakdown at the lipid or lipid/protein junctions)

Ex:- Enzyme urease has been succesfully entrapped.

Method is expensive

Do not bite the hands that feeds you.



Endocytosis:-

Intracellular vesicles could be induced in erythrocytes containing small molecules, drugs or virus from external medium.

↓

Method is efficient for loading large particles such as viruses (upto 100 nm dia), enzymes and small molecules.

Ex Entrapment of glucose, Insulin and β-glucuronidase by a chlorpromazine induced endocytosis.

4) Membrane perturbation:-

Antibiotics such as amphotericin-B damage microorganism by ↑ing the permeability of their membrane to metabolites and IONS.

Ex Amfotericin-B has used to Load erythrocytes with antileukaemic drug daunomycin.

Amfotericin B reacts ∵ Cholesterol of cell membrane and pulled pores.

(5) Normal transport mechanism:-

Loading of drug without disrupting the erythrocyte membrane, by Incubating the drug and erythrocytes for varying period of time.

(6) Lipid fusion method.

Fused lipid vesicle containing inositol hexaphosphate with human erythrocytes.

↓

The Incorporated Inositol hexaphosphate in erythrocytes provided a significant lowering of the O_2 affinity for hemoglobin in intact erythrocytes.

DisAdv:- Low encapsulation efficiency.

Ex Encapsulation of tyrosine kinase into human erythrocytes.

Characterisation of Resealed erythrocytes:-

(a) Drug content Determination:-

Packed loaded cells are deproteinized with acetonitrile after centrifugation at 3000 rpm for fixed time interval.

* the clear supernatent is assayed for drug content.

b) In-vitro drug release and haemoglobin content:-

The cell suspension (5% hematocrit in PBS) are stored at 4°C in amber colored glass containers.

Periodically the clear supernatent are withdrawn using a hypodermic syringe equipped with 0.45μ filter

↓

Deproteined by using methanol and were estimated for drug content.

↓

The supernatent of each sample after centrifugation is collected and assayed for % hemoglobin release may be calculated by using

% Hemoglobin release = $\frac{A_{540} \text{ of sample} - A_{540} \text{ of blank}}{A_{540} \text{ of } 100\% \text{ hemoglobin}}$

Mean corpuscular hemoglobin

$[MCH\ (pg)] = \frac{\text{Hemoglobin (g/100 ml)} \times 10}{\text{Erythrocyte count (millions/cumm)}}$

A_{540} → Absorbance at 540 nm.

c) o/o cell recovery:-

By counting the no. of intact cells per cubic mm of packed erythrocytes before and after loading the drug.

a) Morphology:-

Phase contrast or electron microscopy of normal and loaded erythrocytes.

d) Osmotic fragility:-

Reliable parameter for the in-vitro evaluation of carrier erythrocytes with respect to shelf life, in-vivo survival and the effect of encapsulated substances.

Normal and loaded erythrocytes of drugs are incubated seperately in stepwise decreasing % of NaCl soln (0.9 to 0.1 %) at 37±2°C for 10 min.

Then centrifuge at 2000 rpm for 10 min and supernatent examined for drug and hemoglobin content.

It is based on resistance of cells to hemolysis in diff conc of hypotonic saline.

e) Osmotic Shock:-

Erythrocyte susp. (1ml 10% hct) were diluted with distilled water (5ml) and centrifuged at 3000 rpm for 15 min.

The supernatent was estimated for % hemoglobin release by UV.

g) Turbulence Shock:-

Measure of simulating destruction of loaded cells during injections.

Normal and drug loaded cells are passed through 23 gauge hypodermic needle at flow rate of 10 ml/min (flow rate of blood). → collection of an aliquot and centrifugation at 2000 rpm for 10 min.

The Hb in withdrawn sample is estimated.

b) Determination of entrapped magnetite:—

Atomic absorption Spectroscopic method to determine magnetite conc^n.

The hydrochloric acid is added to a fixed amount of magnetite bearing erythrocytes and contents are heated at 60°C for 2h.

* Then 20% W/v trichloroacetic acid is added and supernatant obtained after centrifugation is used to determine magnetite conc^n using atomic absorption spectroscopy.

(2) ESR:-

Estimate suspension stability of red blood cells in plasma and is related to number, size of red cells and conc^n of plasma protein (fibrinogen and α & β globulins).

↓

Measured in ESR tube

↓

Normal blood ESR 0 to 15 mm/hr.

Higher rate is Indication of active but obscure disease processes.

If you wish to reach the highest, begin at the lowest.

(J) Miscellaneous:- — cell size Mean cell vol, energy metabolism rheological properties.

Application of Rescaled erythrocytes:-

① In-vitro application:-

Phagocytosis cells here been used to facilitate the uptake of enzymes by phagolysosomes. In this study enzymes within carrier RBC could be visualized with the help of cytochemical technique

* Glucose 6-Phosphate dehydrogenase (G6PD) delivery can be useful tool for discerning the mechanism that eventually cause these effects.

√ In-vitro application of RBC is that of mediating interaction A protein or nucleic acid to be injected into eukaryotic cells by fusion process.

* When Antibody molecules are introduced using erythrocytic carrier system, they immediately diffuse throughout the cytoplasm. Antibody RBC autoinjected into living cells have been used to confirm the site of action of fragment of diphtheria toxin.

If you lend, you either lose the money or gain an enemy.

In-vivo application:-

a) Targeting of bioactive agents to RE system:-

✓ Revealed erythrocytes with modified surface characteristics (as damaged by heat treatment, glutaraldehyde treatment sulfhydryl reacting agents) are quickly removed from circulation by phagocytic cells located in liver & spleen, suggesting the possibility of use of erythrocytes in targeting of bioactive agents to these cells.

The drug encapsulated erythrocytes have been used for RES targeting in the treatment of following diseases.

(I) Treatment of lysosomal storage disease:-

✓ Revealed erythrocytes have been proposed to deliver lysosomal enzymes to lysosomes of the erythrophagocytic cells, thus resulting in replacement of the missing enzymes (β-glucoronidase, β-galactosidase and β glucosidase in erythrocytes) ✓

→ Enzymes was targeted to liver and most of the activity was concentrated in lysosomes.

(II) Treatment of Gaucher's disease:-

It is due to accumulation of glucocerebrosides derived from catabolized erythrocytes and

leukocytes in spleen, liver and bone marrow macrophages.

SD Glucocerebrosidase encapsulated in erythrocytes.

✓ Two loaded cells survived for 10 days in treated patients and no unwanted reaction were found with respect to blood count, BP and renal function.

(III) Treatment for liver tumors:-

Targeting of anticancer agents to liver for treatment of hepatic carcinomas.

Ex bleomycin, adriamycin, L-asparginase doxrubicin, methotrexate were injected in hepatoma ascites tumors. (hepatic tumor).

(IV) Treatment of parasitic diseases:-

Can be used for targeting of drugs in the treatment of parasitic disease in which the parasite resides in the organ of RES.-

glutaraldehyde treated erythrocytes for liver targeting of an antimaterial agents - primaquine phosphate and an antiamoebic agent, ~~metroda~~ metronidazole.

(v) Removal of RES iron Overload:-

Entrapped desferrioxamine an Iron chelating drug in erythrocyte with a view to promote excretion of Iron in patients with excess body stores. (Body store of iron is present as intracellular ferritin and hemosiderin deposits).

(b) Erythrocytes as circulating carriers:-

It is used as circulating carriers to disseminate bioactive agent for prolonged period of time in circulation.

Ex Various anticancer drugs vitamins, steroids and ~~antibody~~ antibiotics have been encapsulated.

c) Erythrocytes as circulating bioreactors:-

To diminish the level of circulating metabolites. because many substances enter erythrocytes by diffussion.

13 SUNDAY

Ex Efficacy of arginase loaded erythrocytes in reducing plasma arginine level by about 40-1. within 2hr of infusion into a patient with hyperargininemia.

d) In enzyme delivery:-

Hold a true friend with both your hands.

Enzymes can be infused into blood stream to replace missing or deficient enzyme in metabolic disorders.

like Gaucher's disease, hyperargrinaemia, hyper Uricamia.

~~L-Aspar~~

L- Asparaginase has been entrapped in erythrocytes for treatment of leukemia. certain malignant cells are unable to synthesize asparginase and I.V. administration of enzyme L-asparginase lowers the plasma ~~arginine~~ asparagine.

✓ Aminolevulinic acid enzyme used in lead poisoning.

e) prevention of thromboembolism:-

* encapsulated heparin in erythrocytes for prevention of thromboembolism. at the site of thrombus formation.
* Aspirin and ferromagnetic colloid compound loaded red cells to prevent thrombosis.

(f) Drug targeting other than RES.

Encapsulated Urease in the treatment of kidney failure

Enzyme Uricase for the treatment of gout. (high level of Uric acid)

Hospitality must be extended even towards an enemy who comes to your house.