

# Resealed Erythrocytes: A Novel Approach in Targeted Cancer Therapy

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**Abstract**—To explore the various features, drug loading technology, biomedical application of resealed erythrocytes. Resealed Erythrocyte has the ability to move throughout the body, biocompatibility, zero order kinetics of drugs, reproducibility and ease of preparation. Resealed erythrocytes, are an innovative platform in treating cancer, allow for precise drug delivery with very limited systemic toxicity. Engineered RBCs are prepared by making temporary pores in the cell membrane under hypotonic conditions, followed by loading therapeutic agents such as chemotherapeutics (L-asparaginase, sorafenib), genetic materials, or nanoparticles, and finally resealing the cells to retain their membrane integrity. With their biocompatibility, biodegradability, and long-circulating time, resealed erythrocytes have huge potential to overcome all the problems associated with traditional cancer therapy. More recent work has been towards surface modification techniques, which involve conjugation with tumour-specific ligands, monoclonal antibodies, or peptides, enabling cancerous tissues to be targeted with specificity. Magnetic guidance systems incorporate nanoparticles within erythrocytes for external control, using magnetic fields to direct the drug-loaded cells to target tumour sites. In addition, bio responsive systems release their therapeutic payloads in response to various stimuli, such as pH or enzymes, present in the tumour microenvironment, to facilitate drug action. Integration of resealed erythrocytes with some of the more advanced technologies, such as AI. While challenges in scalability, regulatory approval, and storage stability remain, resealed erythrocytes hold transformative potential in cancer therapy, ushering in a new epoch in precision medicine that promises not only safer but also more effective solutions in the fight against cancer.

**Index Terms**—resealed erythrocytes, targeted cancer therapy, drug delivery, tumour-specific targeting, precision medicine.

## I. INTRODUCTION

Cancer is one of the main causes of mortality worldwide, and conventional chemotherapy often suffers from limitations such as poor bioavailability, non-specific targeting, and severe side effects. Targeted drug delivery systems, such as resealed erythrocytes, offer a solution to these challenges by delivering therapeutic agents directly to cancer cells while minimizing damage to healthy tissues. Resealed erythrocytes are RBCs that have been loaded with drugs and resealed to retain their structural and functional integrity. This review discusses the potential of resealed erythrocytes in cancer therapy, with examples of their application in delivering sorafenib, Adriamycin, L-asparaginase. [1-4]

## II. 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<sub>2</sub>) to the body tissues via the blood flow through the circulatory system.[2]

### COMPOSITION OF ERYTHROCYTES:

- Blood contains about 55% of fluid portion (plasma) 45% of corpuscles or formed elements.
  - Normal blood cells have extensible, elastic, biconcave and non-nucleated configuration with a diameter ranging from 6-9 microns and the thickness is nearly 1-2 microns.
  - Erythrocytes have a solid content of about 35% most of which is Hb and rest 65% being water.
- [1]

LIFE SPAN: 120 days

SOURCE OF ERYTHROCYTES: Different mammals' blood is extracted and their erythrocytes

have been used for drug delivery, including mice, rats, rabbits, monkeys, chicken and sheep. [1]

**ISOLATION OF ERYTHROCYTES:**

- Blood is taken in a heparinized tubes by vein-puncture.
- Blood is drawn by making a cardiac/splenic puncture (in small animal) and through veins (in large animals) in a syringe containing a drop of anti-coagulant.
- The blood sample is centrifuged at 2500rpm for 5min at  $4 \pm 10^{\circ}\text{C}$  in a refrigerated centrifuge.
- except packed cells the other contents are carefully removed and washed thrice with phosphate buffer

saline (pH=7.4) and then they are diluted with same buffer and stored at  $40^{\circ}\text{C}$  for as long as 48hrs before use. [2-3]

**RESEALED ERYTHROCYTES:** For drug loaded carrier erythrocytes are made by taking blood samples from the suitable organism, isolating erythrocytes from plasma, encapsulating the drug in those erythrocytes and resealing them. Therefore, these are known as resealed erythrocytes. The whole process is based on the reciprocation of these cells under osmotic conditions. [4]

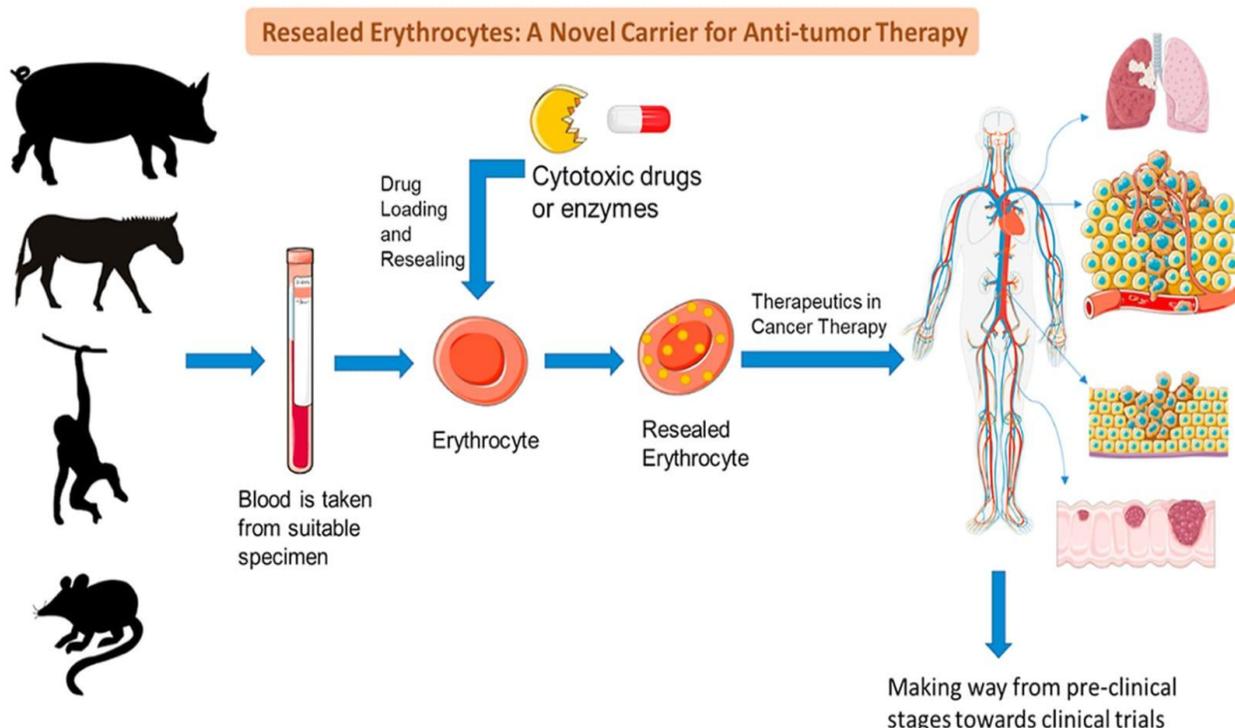


Fig1-Resealed Erythrocytes: A Novel Carrier for Anti-tumour Therapy [11]

**ERYTHROCYTES CAN BE USED AS CARRIERS IN TWO WAYS:**

1. Targeting particular tissue/organ: In this method, the cellular contents of RBC are removed completely only leaving its membrane intact. So, the drug is encapsulated into the cell and resealed with the help of resealing buffer. These are known as red cell ghosts.
2. For continuous or prolonged release of drugs: Many methods are available for entrapping of drugs within erythrocytes. They remain in the systemic

circulation a long time (till 120 days) and release the drug at zero order kinetics.[4]

**EVALUATION OF RESEALED ERYTHROCYTES:**

1. Drug content quantification
2. Percent cell recovery and Morphological study
3. Entrapped magnitude study
4. Shelf life, Stability and Cross linking of Released Erythrocytes
5. Particle size and zeta potential analysis
6. Osmotic fragility and Osmotic shock study
7. Turbulence shock study
8. Erythrocyte sedimentation rate (ESR) [2]

Table 1: METHODS USED FORENTRAPMENT OF DRUG IN ERYTHROCYTES:

| METHOD                    | PRINCIPLE  | HAEMATO CRIT VALUE | EFFICIENCY | ADVANTAGES   | LIMITATIONS   | REFERENCE |
|---------------------------|--|--------------------|------------|--|---|-----------|
| 1.Hypotonic Dilution      | Swell erythrocytes in hypotonic solution to form pores, then reseal in isotonic solution.                | 30-50%             | 20-50%     | 1.Suitable for small molecules<br>2. simple, cost effective  | Low encapsulation efficiency, risk of haemolysis        | [1-5]     |
| 2. Hypotonic Dialysis     | Dialyze erythrocytes against hypotonic buffer to create pores, then reseal in isotonic conditions        | 80%                | 30-60%     | 1.Better control over pore size<br>2. High efficiency        | Time consuming, Requires specialized equipment          | [1-5]     |
| 3.Electroporation         | Apply electric pulses to create temporary pores for drug entry, which reseal naturally after the process | 30-50%             | 20-40%     | High efficiency, suitable for large molecules                | Risk of cellular damage, requires specialized equipment | [1-5]     |
| 4.Membrane Perturbation   | Use chemicals to disrupt the membrane, form pores, and encapsulate the drug during resealing             | 30-50%             | 20-40%     | Simple and effective for certain drugs                       | Toxicity of chemicals, risk of cell damage              | [1-5]     |
| 5.Hypotonic osmotic lysis | Lyse erythrocytes osmotically to create space for the drug, then reseal in isotonic solution             | 40-60%             | 50-80%     | High efficiency, suitable for both small and large molecules | Requires careful control of osmotic conditions          | [1-5]     |
| 6. Endocytosis            | Internalize drugs into erythrocytes via natural endocytosis without significant membrane disruption      | 30-50%             | 10-30%     | Non-invasive, maintains cell integrity                       | Low efficiency, limited to specific drugs               | [1-5]     |

|                 |   |           |        |  |   |       |
|-----------------|---|-----------|--------|--|---|-------|
| 7. Pre-swelling | Pre-swell erythrocytes in hypotonic solution to form pores, load the drug, reseal in isotonic condition | 30-50%    | 30-60% | Improved efficiency, simple process          | Risk of haemolysis, requires optimization         | [1-5] |
| 8. Lipid Fusion | Erythrocytes fused with lipid vesicles to encapsulate drugs or biomolecules                             | Above 50% | 1%     | Improves oxygen transport & Biocompatibility | Potential instability of lipid erythrocyte fusion | [3]   |

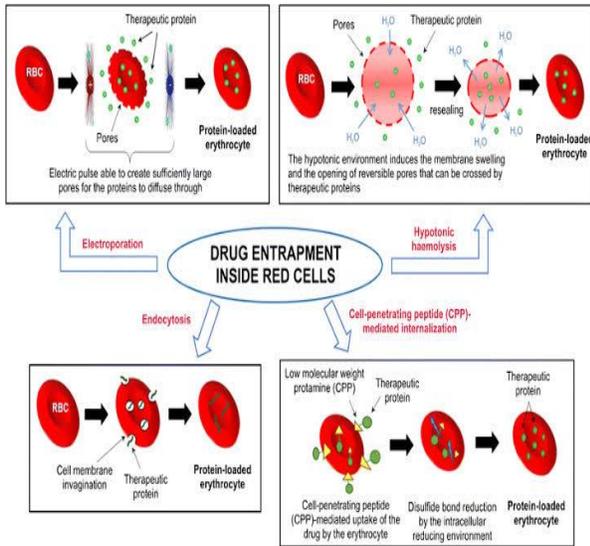


Fig:2 Electroporation, Hypotonic Haemolysis,

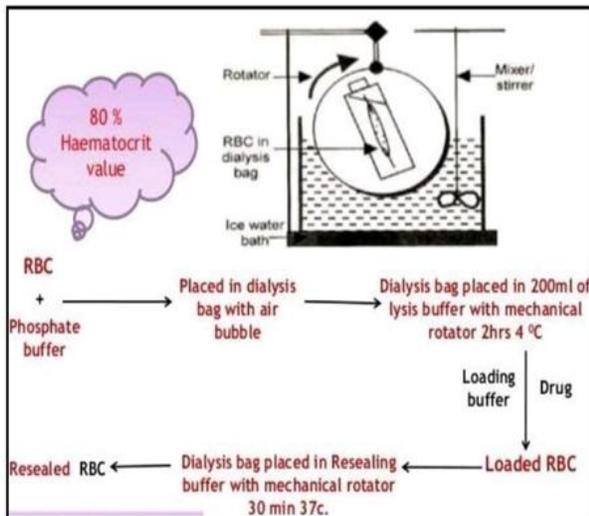


Fig:3 Dialysis method [1]

Endocytosis, Cell- Penetrating Peptide Mediated Internalization Methods

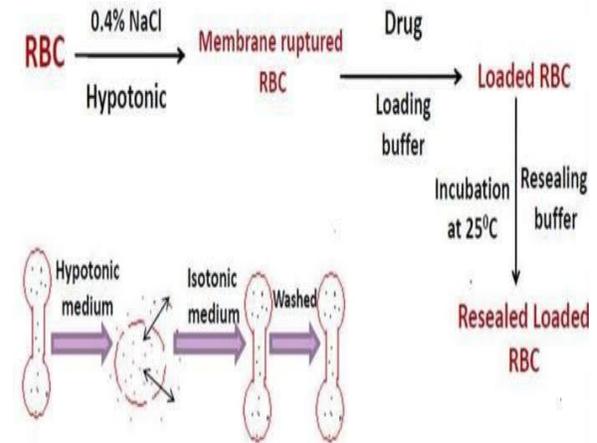


Fig:4 Dilution method [1]

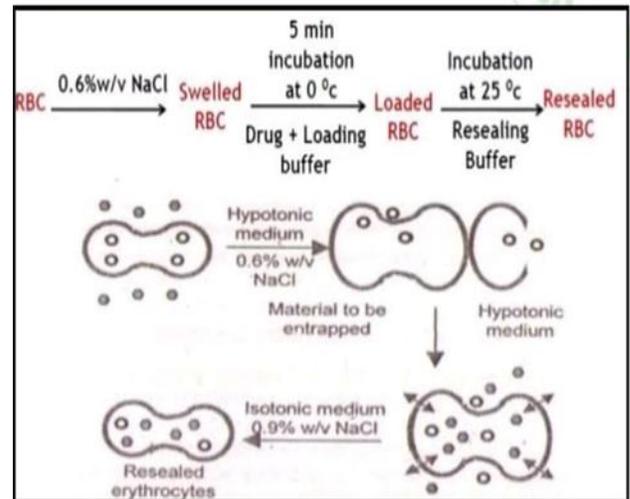


Fig:5 Hypotonic Pre- swell Method [1]

### III. APPLICATIONS

#### 1. Sorafenib

Sorafenib is a multi-kinase inhibitor used in the treating hepatocellular carcinoma. However, its poor solubility and systemic toxicity limit its clinical application. Encapsulation of sorafenib within resealed erythrocytes can improve its solubility and target it to tumour tissues, reducing off-target effects. Studies have shown that sorafenib-loaded erythrocytes exhibit enhanced antitumor activity and reduced cardiotoxicity compared to free sorafenib. [6] Study: It aims to formulate and characterize sorafenib-loaded resealed erythrocytes (SoRE) and

investigate their anticancer activity in a rat model of hepatocellular carcinoma.

Method: SoRE were prepared by hypotonic dialysis of red blood cells obtained from Wistar rats using a range of drug-containing dialysis mediums (2-10 mg/ml) and osmosis time (30-240 mins). Optimized SoRE (8 mg/mL and 240 mins) were characterized for size, morphology, stability, entrapment efficiency, in vitro release profiles, and in vivo efficacy evaluations. For efficacy studies, optimized SoRE were intravenously administered to Wistar rats having hepatocellular lesions induced by aflatoxin B and monitored for in vivo antineoplastic activity. [6]

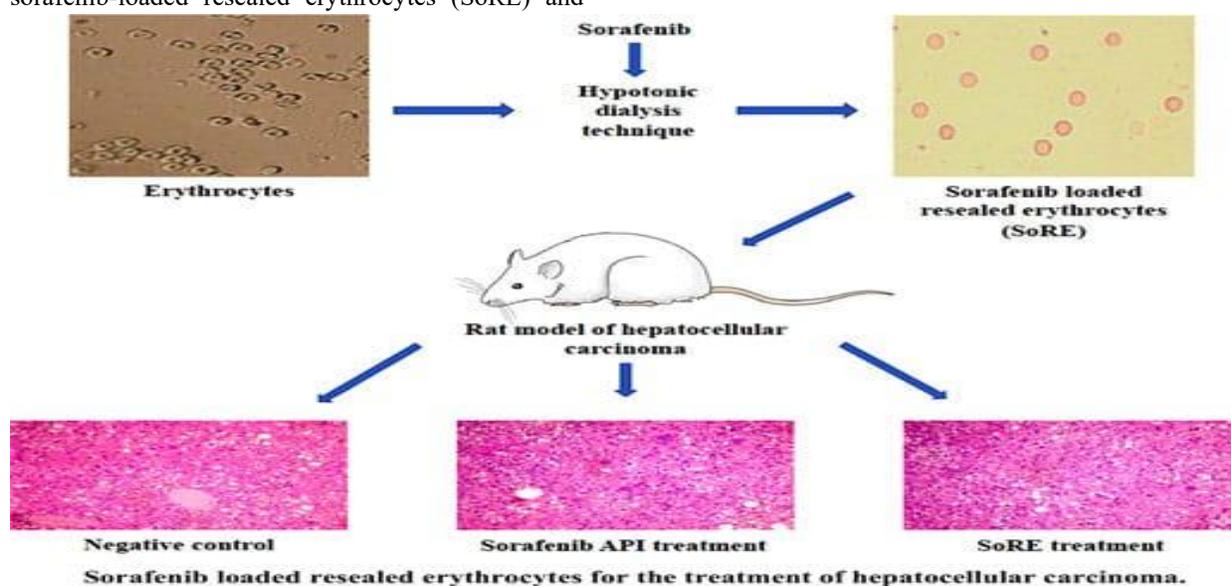


Fig6: Sorafenib loaded resealed erythrocytes for the treatment of hepatocellular carcinoma [6]

Results: The amount of sorafenib entrapped was directly proportional to the drug concentration in the dialysis medium and duration of osmosis; highest for 10 mg/mL and 240 minutes and lowest for 2 mg/mL and 30 minutes, respectively. Optimized SoRE were biconcave with a size of 112.7 nm and zeta potential of  $-11.95 \pm 2.25$  mV. Osmotic and turbulence fragility were comparable with native erythrocytes.

Conclusion: Drug release follows the first-order pattern. In vivo investigations reveal better anticancer activity of SoRE formulation compared to sorafenib standard preparation. Resealed erythrocytes loaded with sorafenib displayed first-order in vitro release and promising anticancer activity in a rat model of hepatocellular carcinoma.[6]

#### 2. L-Asparaginase

L-Asparaginase is used in the treating ALL. It works by depleting asparagine, an essential amino acid for cancer cells. However, its immunogenicity and short half-life limit its efficacy. Encapsulations of L-asparaginase within resealed erythrocytes have shown to prolong its circulation time and reduce immunogenicity, enhancing its therapeutic potential. [7-8, 13]

Mechanism of Action:

The method used is hypotonic dialysis, where erythrocytes are loaded with L-asparaginase and then resealed to restore their original biconcave shape. These engineered erythrocytes act as bioreactors, releasing the enzyme gradually into the bloodstream

while protecting it from immune recognition and degradation. [7-8]

Another method that can be used is endocytosis.[9]

Mean dose: 100 IU/kg [9]

Advantages:

1. Prolonged circulation time: Encapsulation in erythrocytes extends the half-life of L-ASNase
- 2.Reduced immunogenicity: The erythrocyte membrane shields the enzyme from the immune system, lowering the risk of allergic reactions.
- 3.Targeted delivery: Erythrocytes can passively target the reticuloendothelial system, which is beneficial in treating leukemic cells residing in these tissues.
- 4.Minimized toxicity: Encapsulation reduces systemic exposure to free L-asparaginase, decreasing side effects such as hepatotoxicity and pancreatitis.
- 5.Clinical Trials: Several preclinical and clinical studies have investigated the efficacy and safety of L-asparaginase-loaded erythrocytes:

Phase I/II Clinical Trials:

A Study [14] demonstrated that the feasibility and safety of L-asparaginase-loaded erythrocytes in patients with ALL. The study reported prolonged enzyme activity in the bloodstream and reduced immunogenicity compared to free L-asparaginase.

Another Clinical trial [15] evaluated that the pharmacokinetics and pharmacodynamics of erythrocyte-encapsulated L-asparaginase in paediatric ALL patients. The results showed improved therapeutic efficacy and reduced adverse effects.

Phase III Clinical Trials:

A large-scale Phase III trial [16] compared the efficacy of erythrocyte-encapsulated L-asparaginase with the standard formulation. The trial confirmed the superior safety profile and comparable efficacy of the encapsulated form.

3. Adriamycin

Adriamycin also known as doxorubicin, is a widely used anthracycline antibiotic with potent antitumor activity. It is effective against a variety of cancers, including breast cancer, leukemia, lymphoma, and sarcomas. However, its clinical use is limited by severe side effects, such as cardiotoxicity, myelosuppression, and dose-dependent cumulative toxicity. Adriamycin (doxorubicin) was entrapped in erythrocytes by using dialysis technique which includes transient hypotonic haemolysis followed by isotonic resealing. [10,11]

Dose: Up to 1.6 mg/ml of the packed RBC's and its efficiency is 60-80%.

Mechanism of Action:

Doxorubicin exerts its anticancer effects by intercalating into DNA, inhibiting topoisomerase II, and generating free radicals, which causes DNA damage and apoptosis in cancer cells. When encapsulated in resealed erythrocytes, the drug is protected from rapid clearance and degradation, allowing for controlled and sustained release. [10, 11]

Advantages of Erythrocyte Encapsulation:

- 1.Reduced toxicity: Encapsulation minimizes systemic exposure to free doxorubicin, reducing cardiotoxicity and other side effects.
- 2.Prolonged circulation: Erythrocytes extend the half-life of doxorubicin, enhancing its therapeutic window.
- 3.Targeted delivery: Erythrocytes can passively accumulate in the reticuloendothelial system (RES) and tumour tissues due to enhanced permeability and retention (EPR) effects.
- 4.Improved pharmacokinetics: Encapsulation improves the biodistribution of doxorubicin, increasing its concentration in tumour tissues while decrease other side effects.

Preclinical Studies:

A Study [17] demonstrated the feasibility of encapsulating doxorubicin in erythrocytes. The study showed that erythrocyte-encapsulated doxorubicin retained its anticancer activity while reducing cardiotoxicity in animal models.

Another Study [18] explored the utilization of erythrocytes as carriers for doxorubicin in treating solid tumours. The results indicated improved tumour targeting and reduced systemic toxicity compared to free doxorubicin.

Animal models:

A Research [19] evaluated the efficacy of erythrocyte-encapsulated doxorubicin in murine models of leukemia. The study reported prolonged survival and reduced cardiotoxicity in treated animals.

Clinical Trials:

Phase I/II Clinical Trials:

A Phase I clinical trial [20] investigated the efficiency, efficacy, pharmacokinetics of erythrocyte-encapsulated doxorubicin in people who are suffering

with advanced solid tumors. The trial demonstrated reduced cardiotoxicity and improved tolerability compared to conventional doxorubicin.

A Phase II clinical trial [21] evaluated the efficacy of erythrocyte-encapsulated doxorubicin in patients with metastatic breast cancer. The study reported comparable antitumor activity with significantly fewer side effects.

#### IV. ADVANTAGES OF RESEALED ERYTHROCYTES:

##### 1. Natural Stealth Properties:

Erythrocytes evade immune surveillance due to their natural presence in the bloodstream, making them ideal for delivering drugs without triggering immune responses.

##### 2. Ability to Bypass Biological Barriers:

Resealed erythrocytes can traverse biological barriers (e.g., blood-brain barrier) more effectively than synthetic carriers, enabling targeted delivery to hard-to-reach tissues.

##### 3. Tumor Microenvironment Targeting:

Erythrocytes can exploit the leaky vasculature of tumours to passively accumulate in cancerous tissues, enhancing drug delivery to tumours.

##### 4. Carrier for Oxygen Delivery:

Resealed erythrocytes are also used for delivering oxygen to hypoxic tumour regions, improving the efficacy of radiation therapy and chemotherapy.

##### 5. Dual Functionality:

Erythrocytes can simultaneously deliver therapeutic agents and perform their natural function of oxygen transport, making them multifunctional carriers.

##### 6. Minimal Toxicity:

Unlike synthetic nanoparticles, resealed erythrocytes do not introduce toxic materials into the body, reducing the risk of systemic toxicity.

##### 7. Potential for Combination Therapy:

Multiple drugs or therapeutic agents (e.g., chemotherapeutic drugs, immunomodulators, or imaging agents) can be co-encapsulated within erythrocytes for synergistic effects.

##### 8. Natural pH Sensitivity:

Erythrocytes can emit the drug in response to the acidic pH of the tumour microenvironment, enabling site-specific drug release.

##### 9. Reusability of Erythrocytes:

Erythrocytes can be harvested, processed, and reused for multiple drug-loading cycles, making them a sustainable option. [1-4]

#### V. DISADVANTAGES OF RESEALED ERYTHROCYTES:

##### 1. Limited Encapsulation of Large Molecules:

While small molecules can be easily encapsulated, large molecules (e.g., proteins, nucleic acids) face challenges due to size restrictions and membrane permeability.

##### 2. Batch-to-Batch Variability:

The preparation of resealed erythrocytes can lead to variability in drug-loading efficiency, release kinetics, and cell integrity, making standardization difficult.

##### 3. Risk of Clogging in Capillaries:

Resealed erythrocytes, especially if damaged or aggregated, can clog small blood vessels, leading to microcirculation issues.

##### 4. Ethical Concerns with Donor Blood:

The use of donor erythrocytes raises ethical concerns, particularly in regions with limited blood supply or cultural sensitivities.

##### 5. Limited Flexibility in Drug Release Profiles:

Achieving rapid or pulsatile drug release from resealed erythrocytes is challenging, as their natural design favours slow and sustained release.

##### 6. Sensitivity to External Conditions:

Resealed erythrocytes are sensitive to temperature, pH, and mechanical stress, requiring strict storage and handling conditions.

##### 7. Potential for Drug Leakage:

Encapsulated drugs may leak out prematurely during circulation, reducing the effectiveness of targeted delivery.

##### 8. Complex Quality Control:

Ensuring the quality, stability, and functionality of resealed erythrocytes requires sophisticated analytical techniques, increasing the complexity of production.

##### 9. Limited Applicability for Certain Diseases:

Resealed erythrocytes are not appropriate for diseases requiring rapid drug action or those involving erythrocyte dysfunction (e.g., sickle cell anaemia). [1-4]

Table 2: OVERVIEW:

| ASPECTS                | DETAILS  | REFERENCES |
|------------------------|--|------------|
| Advantages             | <ul style="list-style-type: none"> <li>-Biocompatible and biodegradable.</li> <li>- Prolonged circulation time.</li> <li>- Natural targeting to organs like the liver and spleen.</li> <li>- Reduced immunogenicity.</li> <li>- Ability to encapsulate both polar and non-polar drugs.</li> </ul>  | [1-4]      |
| Applications In Cancer | <ul style="list-style-type: none"> <li>-Targeted delivery of chemotherapeutic agents.</li> <li>- Delivery of immunomodulators for cancer immunotherapy.</li> <li>- Enzyme replacement therapy in cancer metabolism.</li> <li>- Diagnostic imaging agents for tumour detection.</li> <li>- Delivery of gene-editing tools (e.g., CRISPR).</li> </ul>      | [1-4]      |
| Challenges             | <ul style="list-style-type: none"> <li>-Low drug-loading capacity.</li> <li>- Risk of haemolysis during encapsulation.</li> <li>- Limited scalability for industrial production.</li> <li>- Stability issues during storage.</li> <li>- Difficulty in controlling drug release kinetics.</li> </ul>  | [1-4]      |
| Targeting Mechanisms   | <ul style="list-style-type: none"> <li>-Passive targeting via Enhanced Permeability and Retention (EPR) effect.</li> <li>- Active targeting using surface-modified erythrocytes with ligands (e.g., antibodies, peptides).</li> <li>- Natural tropism to the reticuloendothelial system (RES).</li> </ul>  | [1-4]      |
| Drug Release Kinetics  | <ul style="list-style-type: none"> <li>-Controlled release via modulation of erythrocyte membrane properties.</li> <li>- pH-sensitive release in the tumour microenvironment.</li> <li>- Enzyme-triggered release in specific tissues.</li> </ul>  | [1-4]      |
| Clinical Progress      | <ul style="list-style-type: none"> <li>-Preclinical studies show promising results in animal models.</li> <li>- Limited clinical trials due to regulatory and scalability challenges.</li> <li>- Potential for personalized cancer therapy.</li> </ul>   | [1-4]      |
| Future Perspectives    | <ul style="list-style-type: none"> <li>-Development of hybrid systems combining erythrocytes with nanoparticles.</li> <li>- Use of genetically engineered erythrocytes for enhanced targeting.</li> <li>- Exploration of exosome-like vesicles derived from erythrocytes.</li> <li>- Integration with AI for optimized drug delivery systems.</li> </ul> | [1-4]      |
| Key Examples           | <ul style="list-style-type: none"> <li>-Doxorubicin-loaded erythrocytes for breast cancer.</li> <li>- Methotrexate-loaded erythrocytes for leukaemia.</li> <li>- Erythrocytes can be used as carriers for cancer vaccines.</li> </ul>  | [1-4]      |
| Advancements           | <ul style="list-style-type: none"> <li>-Nanoerythroosomes, Erythroosomes</li> <li>-Industrial Purpose: Red cell loader, CPPs, DMSO, Enzyme loaded erythrocytes, Magnetic RE, Stem cell derived erythrocytes, genetically modified erythrocytes.</li> </ul>   | [1,13]     |

## VI. CONCLUSION

Resealed erythrocytes represent a ground-breaking advancement in targeted cancer therapy, offering a biocompatible, biodegradable, and efficient drug delivery system. By leveraging the natural properties of erythrocytes, this approach ensures prolonged circulation, sustained drug release, and reduced

systemic toxicity. The integration of tumour-specific targeting, magnetic guidance systems, and bio responsive drug release mechanisms further enhances the precision of treatment while reducing side effects. Despite their immense potential, challenges such as large-scale production, regulatory approvals, and storage stability must be addressed before widespread clinical implementation. Ongoing research in

nanotechnology, artificial intelligence, and bioengineering holds promise for overcoming these barriers, making resealed erythrocytes a viable and revolutionary tool in precision medicine. With continued advancements, this novel approach has the ability to redefine cancer therapy, offering safer and more effective treatment solutions for patients worldwide.

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