Review on RBC a Natural Model for Advanced/ Targeted Drug Delivery System for Anti-cancer Therapy: Potential and Challenges

By

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A thesis submitted to the School of Pharmacy in partial fulfilment of the requirements for the degree of Bachelor of Pharmacy

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Declaration

It is hereby declared that

1. The thesis submitted is a literature review done by myself while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
4. I have acknowledged all main sources of help.

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Approval

The thesis titled “Review on RBC a Natural Model for Advanced/ Targeted Drug Delivery System for Anti-cancer Therapy: Potential and Challenges” submitted by Maimuna Zahed Shaon (18146018) of Spring 2018, has been accepted as satisfactory in partial fulfilment of the requirement for the degree of Bachelor oh Pharmacy (Hons.).

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Ethics Statement

This study does not involve any human and animal trial.

Abstract

Red blood cell loaded therapeutic agents have been acknowledged because of its advantageous biologic properties. Among all the drug carriers, red blood cell is being a unique nano drug carrier and so considered as a vascular drug delivery system after surface modification. This review is based on anti-cancer drug carried by RBC and its tremendous potentials and promising feature for encouraging development for future drug delivery system. Hence, the resealed erythrocyte and camouflaged nanoerythrosomes are the engineered RBC particles which are considered as targeted chemotherapeutic drug carrier. This review is also focused on the comparison between cancer drugs loaded with RBC and other nano carriers. Furthermore, several successful clinical trials for drugs such as doxorubicin, daunorubicin, l-asparaginase, methotrexate, 5-fluorouracil are specifically focused in this review which are loaded into RBC as chemotherapeutic agent. However, few limitations and drawbacks are noticeable which need to be solved before loading drug into RBC.

Keywords: Drug Carrier; Targeted drug deliver; Resealed erythrocyte; Nanoerythrosomes; Encapsulation; Chemotherapy;

Dedication (Optional)

Dedicated to my beloved parents and honourable supervisor Sir for giving me support and motivation during this thesis.

Acknowledgement

Firstly, I would like to express my earnest gratitude to Almighty Allah, who has provided me the rational power, wisdom, patience and strength to come up to this level. His blessings have made me to reach to this far. His continuous mercy upon me has helped me to follow my goal and dream.

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List of Acronyms

SIRP	Signal-regulatory protein alpha
NIR	Near-infrared spectroscopy
LbL	Layer by Layer
HRP	Homologous restricted protein
MTX	Methotrexate
MCP	Membrane cofactor protein
DAF	Decay acceleration factor
CR 1	Complement receptor

Chapter 1

Introduction

1.1 Drug Delivery System

The method of administration of a medicine can have a substantial influence on its efficacy. It is now feasible to more effectively manage the pharmacokinetics, pharmacodynamics, toxicity, immunogenicity, and effectiveness of medications through the presence of a variety of drug delivery systems (DDSs). By determining the most appropriate delivery method for a particular medicinal molecule, one may "optimize" the medicine's performance within the body(December & Lansdowne, 2020).

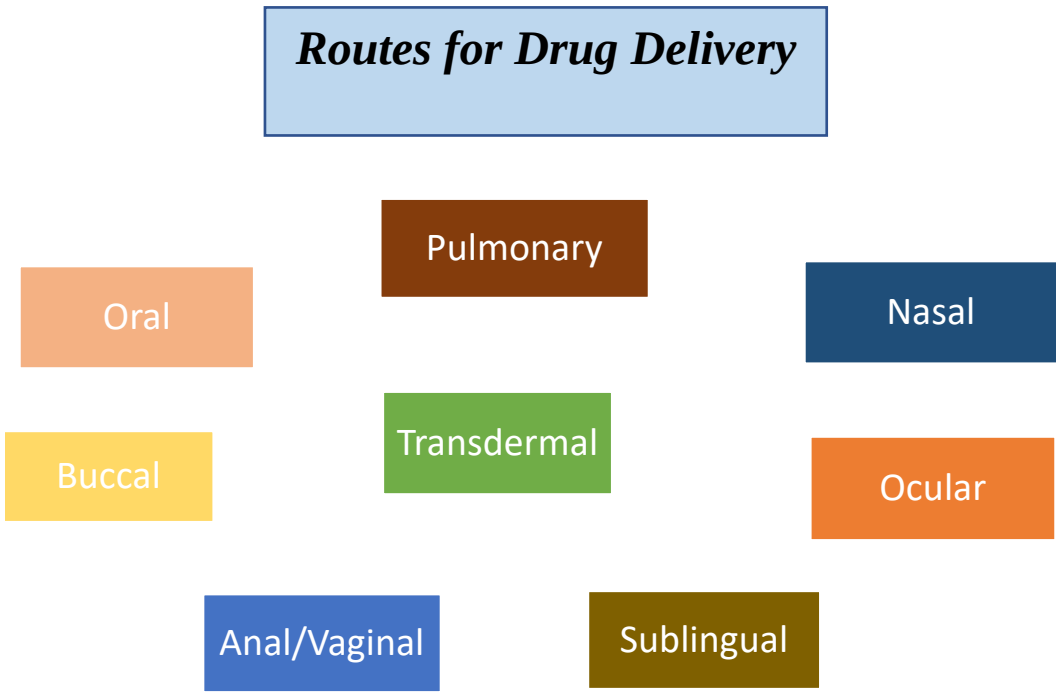

Figure 1: List of the routes for drug delivery

Despite this improvement, many drugs, including those found utilizing cutting-edge molecular biology methodologies, have undesirable side effects as a result of the drug

interaction with healthy cells which are not the primary target. Side effects impair our capacity to develop the most effective drugs for a variety of disorders, including cancer, neurological diseases, and communicable diseases. Drug - delivery systems regulate both the rate of drug release and the site of drug release in the body. Certain systems are capable of doing both functions(NIH, 2016).

1.2 Conventional Drug Delivery Routes and Their Limitations

Table 1: Characteristics of conventional drug delivery routes with their limitations

Drug Delivery System	Characteristics	Limitations
Oral	• non-invasive, easy usability, cheap, and the can absorb highly in the gastrointestinal (GI) tract (Indurkhya et al., 2018).	• strictly limited delivery system for paediatric, geriatric, and cognitively impaired patients (Indurkhya et al., 2018)
Buccal	• avoids first-pass effects • has lipophilicity • required in extended-release drug delivery formulations • attachment to the mucosa are typically preferred(Sudhakar et al., 2006).	• quite challenging for drug absorption, • at present, delivery of bulkier biopharmaceuticals is confined to small molecule drugs(Sudhakar et al.,

		2006).
Pulmonary	• an efficient treatment method localized lung illness • has a large adsorption surface area and a high permeability alveolar membrane; • may be used as an administration route for systemic disorders. • insensitive to dietary problems and interpatient metabolic variability (Ali, 2010).	• the mucus' barrier properties and the drug–mucus interactions. • mucociliary evacuation shortens the duration of drug retention in the lungs, which may impair the pharmacological effectiveness of slowly absorbed medicines (Ali, 2010).
Ocular	• a challenging issue for drug delivery experts, because the eye's unique anatomy and physiology prevent medications from being absorbed via the eye. • static, dynamic, and metabolic ocular barriers all obstruct absorption of the drug through the eye(Patel et al., 2013).	• constrained drug delivery by a variety of precorneal, dynamic, and static ocular barriers. • therapeutic drug levels really aren't sustained in target tissues for an extended period of

		time (Patel et al., 2013).
Sublingual	• fast absorption • avoids hepatic first-pass metabolism(Singh et al., 2017)	• decreases the absorption causes by smoking and hence the effectiveness of the drug owing to vasoconstriction of the arteries(Singh et al., 2017).
Nasal	• Used for treatment of local diseases, which affects the upper respiratory tract(*Nasal Drug Delivery Devices: Characteristics and Performance in a Clinical Perspective-a Review Per Gisle Djupesland*, 2012).	• When fast onset is necessary, this administration method can be used to administer small molecule drugs systemically(*Nasal Drug Delivery Devices: Characteristics and Performance in a Clinical Perspective-a Review Per Gisle Djupesland*, 2012).
Transdermal	• non-invasive and feasible for	• the stratum corneum,

	patients who are unconscious or prone to vomit(Prausnitz & Langer, 2008).	which is thick and avascular, are the primary barriers (Prausnitz & Langer, 2008; Singh et al., 2017).

1.3 Recent Drug Delivery Systems and Their Possibilities

Several nanoparticles and biological carrier, computer aided drug delivery system is the main focus of this section.

1.3.1 Smart Nanoparticles

When stimulated, smart nanoparticles release additional drug molecules into the surrounding environment. Physical (heat, light, magnetization, and electricity) chemical (pH, and ions) and biological (enzymes, antibody, and tiny molecules) stimuli are all included in the stimuli(Lee et al., 2015).

1.3.1.1 Polymerics Nanoparticles

Smart or stimuli-responsive polymers are described as those that may respond to external physiochemical stimuli and are employed as nanomedicines. Numerous external stimuli, including as pH, redox, and temperature, have been documented in smart polymeric systems. Polymers' physical/chemical characteristics can proactively change through response to certain stimuli. The rate of drug release can be regulated by the strength of stimuli provided to the manufactured carriers(Alvarez-lorenzo et al., 2014; Filippov et al., 2015). The function of P-glycoprotein (P-gp) can be blocked by pH induced smart polymeric DDSs and

regenerate apoptotic cell signalling pathways(Alvarez-lorenzo et al., 2014; Liu et al., 2016). Additionally, redox sensitive polymer systems have been used to treat intestinal inflammation-related disorders. Temperature-responsive polymers also draw considerable interest because to the phase shift that occurs when the external environment is changed(Liu et al., 2016).

1.3.1.2 Exosomes

Exosomes are nanoparticle-sized membrane vesicles released by specialized cells or organs in response to internal or external stimuli. Exosomes may be targeted to specific organs and keeping biologically active(Dongmei Sun et al., 2013; Van Dommelen et al., 2012). Exosomes can be loaded with a variety of drugs and act as carriers for individualized treatment. Donor cells may transmit exogenous molecules such as proteins, messenger RNAs, microRNAs (miRNAs), and lipids to recipient cells through exosomes, which makes this a suitable drug delivery technique(Haney et al., 2015).

1.3.1.3 Liposomes

Due to their adjuvant properties and targeting capabilities, liposomal delivery of drugs systems is extremely successful in delivering vaccines and genes. They elicit the body's immunological response via antibody creation and repaired gene inputs. Additionally, the mechanism of vaccination and gene delivery is investigated(Gregoriadis & Ryman, 1971). Liposomes safeguard some drugs from biochemical and immunologic degradation, as well as from the action of enzymes. Due to the sustained drug levels in liposomes, toxicity and dosage are reduced. (Rahman et al., 2017).

1.3.1.4 Ligand-Modified Target Drug Delivery Strategy

Ligand-mediated controlled and targeted drug delivery strategies may increase a variety of properties, including permeation over physiological obstacles, penetration into target regions, uptake by target cells, and localization to specific subcellular locations. In the majority of traditional ligand-modified drug carriers, the targeting moieties diffuse evenly throughout the vehicle surface, with just a small fraction interacting with target cells, resulting in low recognition ability and inefficient ligand material consumption. Apart from direct alteration of the vehicle's surface, a novel technique for ligand presentation has been established. The cavity generated by molecular imprinting serves as a recognizing molecule for the target receptors in this strategy(Agarwal et al., 2008; Yao et al., 2008).

1.3.2 3D Printing-Based Drug Delivery Technology

3D printing is indeed a layer-by-layer manufacturing approach that enables the production of three-dimensional delivery of drugs formulas from digital designs. 3D printing is unmatched in its ability to fabricate sophisticated, customised, and on-demand objects. These features are critical in terms of enhancing the safety, effectiveness, and accessibility of medications(Goole & Amighi, 2016; Sandler & Preis, 2016).

1.3.3 Microneedle-Based Transdermal Drug Delivery Technology

The advancement of transdermal medication delivery systems has been substantially advanced by microneedle (MN) technology (TDDS). Due to the microneedle's small size, it may pass the stratum corneum layer of transdermal drug delivery without contacting nerve fibres or blood vessels. As a result, the MN is a painless and easy method of administration, with the capacity to circumvent systemic first pass metabolism and achieve prolonged release(Prausnitz & Langer, 2008; Tucak et al., 2020).

1.3.4 Drug Loaded Erythrocytes Delivery

Drugs injected into erythrocytes are one of the fastest growing and most promising both active and passive target delivery strategies for drugs and enzymes. Erythrocytes are biodegradable and biocompatible, have a long half-life in the circulation, and may be loaded with a wide variety of physiologically active chemicals(Magnani et al., 2012). Targeted medication administration via RBCs can be accomplished in several ways. First, pharmaceuticals can be delivered to the cells of the RES (macrophages), as well as to the liver and spleen, i.e., to the body cells that eliminate old and damaged RBCs. As a result, this strategy may be employed successfully to treat tumours of these tissues. To transfer the drug-loaded erythrocyte into such target cells, the erythrocyte must be changed in such a way that the target cells interpret it as injured. There are numerous techniques for this modification. Each of them results in a change to the erythrocyte membrane. This could be due to the opsonization of RBCs with antibodies directed against their membrane markers (for example, rhesus-antibodies) or the attachment of the complement component C3b to them, as both the Fc fragment of IgG and C3b have surface receptors (Morgan & Harris, 2015). RBCs for medication delivery have a number of advantages over currently used methods and systems. The erythrocyte is a good option for such delivery due to its biocompatibility, biodegradability, extended life in the bloodstream, ability to reduce drug side effects, ease of cellular isolation in large quantities, and scale-up capability (Koleva et al., 2020a).

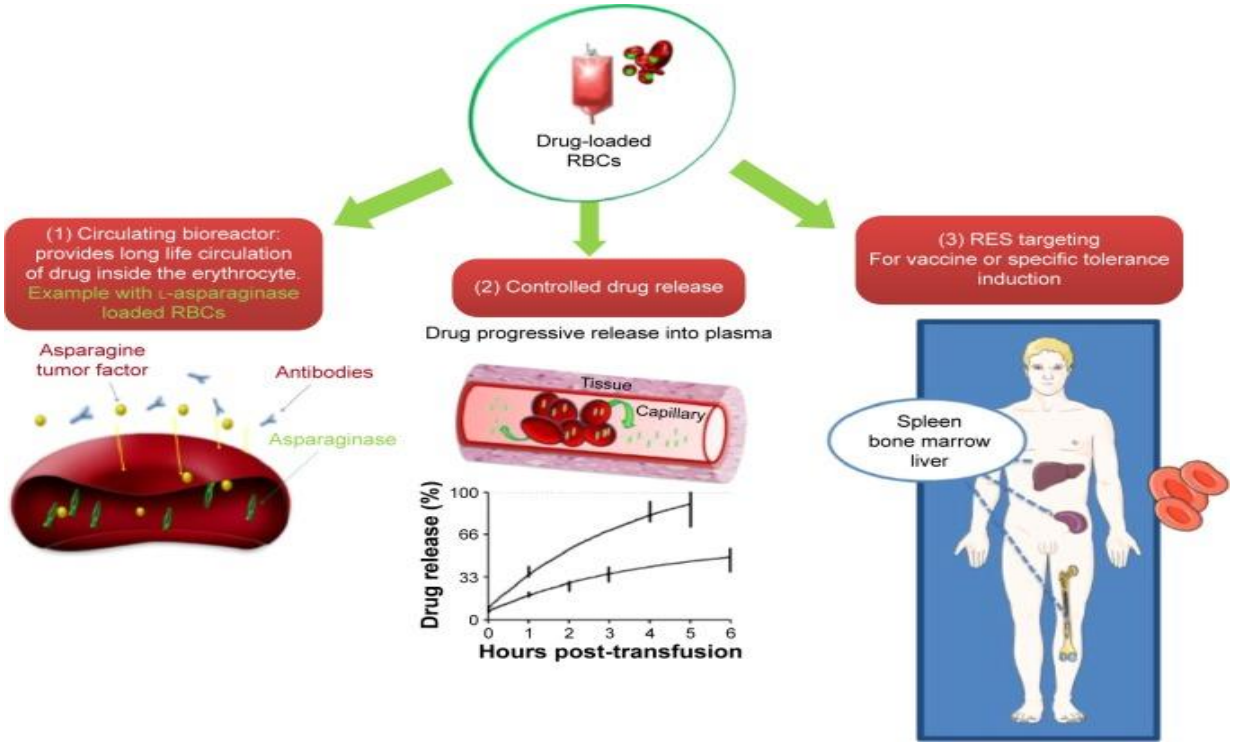

Figure 2: The following diagram illustrates three prospective therapeutic applications for erythro drug carriers: (1) as a circulation bioreactor, (2) for regulated drug release, and (3) for targeting RES (Bourgeaux et al., 2016).

1.3.5 Targeted Drug Delivery

Because very few drugs bind preferentially to their specified therapeutic target, specific targeting strategies are necessary to localize the medication in the appropriate tissue or organ and thereby minimize effectiveness and dose-related harm. The difficulties include selecting the actual target for a particular disease, the appropriate medicine for successful therapy, and secure, disposable drug carriers while minimizing immunostimulatory and nonspecific encounters that are necessary for the body to properly remove foreign material. Additionally, the delivery system's preparation should be straightforward or relatively straightforward, reproducible, and cost-effective. When combined with targeting ligands, nanoparticles (NPs) may be beneficial as carriers of active medicines(Kumar et al., 2017; Maiti & Sen, 2017). To be effective, medication targeting techniques must fulfil two fundamental conditions. After administration, the medications should reach the target areas with minimal dosage and activity loss in the blood circulation. Second, the medications must work exclusively on

target cells while having no adverse effect on healthy tissue. For drug targeting, two methodologies have been used: passive targeting and active targeting(Cho et al., 2008).

1.3.5.1 Passive Targeting

Passive targeting makes use of the target organ's or tissue's inherent circumstances to direct the medicine to the desired location. For instance, passive targeting exploits the specific pathophysiological properties of tumour arteries, namely their leaking vasculature containing 100–800 nm holes, which enables nanodrugs to concentrate in tumour tissues. Additionally, passive targeting is aided by the fact that the microenvironment around tumour tissue is distinct from that of healthy cells(Maiti & Sen, 2017).

1.3.5.2 Active Targeting

Internalization of the targeting conjugates occurs via an endocytosis pathway mediated by the receptor. The designed to target ligands first bind to the receptors, accompanied by the development of an endosome and the encapsulation of the ligand-receptor complex by the plasma membrane. The endosome is subsequently moved to certain organelles, in which acidic pH and otherwise enzymes liberate drugs(Maiti & Sen, 2017).

1.4 Possibilities of Erythrocytes as a Carrier of Drug in Cancer treatment

In general, anticancer drugs loaded into carriers with the primary goal of minimizing their adverse toxicity to the host body continues to hold a great deal of promise for the success of existing cancer therapeutic protocols through efficient drug targeting to the tumour site via passive mechanisms such as enhanced permeability and retention and retention and active mechanisms like antibody targeting (Muzykantov, 2010; Senapati, 2018). RBC encapsulation of L-asparaginase increased enzyme supply to the patient by reducing allergic responses, immunological clearance, and proteolysis (Agrawal et al., 2016). Daunorubicin and

doxorubicin represent two anticancer drugs that were lately reported to be more tolerable when administered via carrier erythrocytes in human trials(Alavi & Varma, 2020; Skorokhod et al., 2007). Furthermore, methotrexate as well as 5-fluorouracil were administered to rats and mice using erythrocyte carriers(Wang et al., 2010; Yuan et al., 2009; Zarrin et al., 2014). The nano erythrosomes were found to be stable and capable of retaining daunorubicin's cytotoxic and chemotherapeutic effect against mouse Leukemia P338-D cells (Javed et al., 2021).

1.4.1 Recent Development

Erythrocytes are now being studied as innately carriers for a variety of intravascular drugs with the purpose of decreasing toxicity, suppressing immunological responses, and providing a prolonged half-life in the blood(Magnani et al., 2012). They can retain a prolonged half-life in circulation if the drug loading procedure has no effect on the membrane's physical characteristics (C. M. J. Hu et al., 2012; Y. W. Wu et al., 2016; Yousefpour & Chilkoti, 2014).

The initial research on erythrocyte-derived drug delivery systems, as well as the initial attempt to use this unique drug delivery system in cancer therapy, was also an enzyme therapy using L-asparaginase (L-ASNase) to treat acute lymphoblastic leukemia (ALL). The anti-cancer effect of L-asparaginase is driven by the fact that certain cancer tissues are unable to synthesize L-asparagine (L-ASN) and must rely on circulating L-ASN to maintain normal physiological purpose (protein synthesis, DNA replication, etc.), whereas normal human tissue can synthesize its own. Thus, elimination of circulating L-ASN via L-asparaginase (L-ASNase) can disrupt cell functioning in some cancer tissues, resulting in cell apoptosis and tumour regression(Mao et al., 2021).

The pharmacologic efficacy of methotrexate-loaded erythrocytes was demonstrated by increases in average survival time in mice having hepatoma ascites tumours. The carrier erythrocytes are exposed to chemicals that stabilize the membrane, primarily glutaraldehyde, which functions by lowering the erythrocytes' deformability. The treated cells are swiftly eliminated from circulation due to liver and spleen identification, and the liver tolerates them well. The glutaraldehyde administration of methotrexate-loaded canine erythrocytes focused the medication specifically to the liver (Ravilla et al., 2012).

Daunorubicin was found to bond significantly better to cleansed erythrocytes than to blood erythrocytes in a study. Daunorubicin's lower equilibrium concentration ratios found in the blood are likely due to a significant amount of daunorubicin binding to plasma proteins and lipoprotein complexes. However, the majority of daunorubicin is bound to erythrocytes (Lin et al., 2019).

For enhanced cancer therapy, a new nanocarrier comprised of red blood cell (RBC)-derived vesicles (RDVs) surface-linked with doxorubicin (Dox) utilizing glutaraldehyde (glu) was studied. Through intravenous injection, the synthesized Dox-gluRDVs displayed superior in vitro cytotoxicity against a panel of cancer cell lines and increased in vivo anticancer activity against subcutaneous melanoma B16F10-bearing mice. Interestingly, an in vitro unique mechanism demonstrated that Dox-gluRDVs' higher cytotoxicity was not due to enhanced cellular uptake but rather to the preference for intracellular distribution of Dox-gluRDVs' released Dox into lysosomes. In contrast to free Dox, Dox-gluRDVs can efficiently deliver Dox into lysosomes, resulting in accumulation of sufficient Dox to fuel mitochondrial ROS overproduction and subsequent loss of mitochondrial membrane potential and apoptotic activation, which accounts for Dox-gluRDVs' superior anticancer activity in vitro and in vivo (S. H. Wu et al., 2021a).

Coating nano-erythrocyte membranes (NEMs) over 5-fluorouracil (5-FU)-loaded liposomes (LPs) was done in another investigation to generate NEM-5-FU-LPs. This framework is utilized to facilitate the rescue of 5-FU-LPs from systemic circulation degradation. NEM-5-FU-LP vesicles, in particular, exhibited a negative charge, a low EE (31%), and adequate stability during a three-week period. SDS-PAGE, phosphatidylserine exposure, and TEM all verified that NEM-5-FU-LPs exhibited erythrocyte membrane mimicry properties. Additionally, when compared to NEM-5-FU, LPs and the 5-FU solution, NEM-5-FU-LPs demonstrated a sustained release profile. The continuous release patterns of NEM-5-FU-LPs resulted in a 72-hour delay in cell survival, indicating a potential for improving in vivo liver cancer targeting (Alqahtani et al., 2019).

1.5 Objectives

- To study the status and ongoing strategies of drug loaded erythrocyte-based delivery system development and
- To analyse some potential candidates of anti-cancers treatment carried by erythrocytes and
- To find out their current limitations and future aspects

1.6 Aim of the Study

The goal of this review paper is the comparative analysis among biological and non-biological drug carriers in which erythrocyte as a camouflaged drug carrier is given emphasize. Here, the study is focused on how the targeted drug delivery for the anti-cancer treatment is promisingly carried by RBC with its modified structure more conveniently. As a result, altering the limitations to the optimum level, tailored drug delivery system by RBC will bring revolutionary treatment method in case of malignancy.

Chapter 2

Methodology

This review paper is based on recent year's research articles with relevance, although few articles were taken from past years to justify the importance of current research of this topic. The articles have been collected from high impact factor journals. Peer reviewed journals, journal manuscripts and original research work were used to enrich this review paper. All of these comprehensive searches have been taken from ResearchGate, Google Scholar, Science Direct, PubMed, etc. The significant journals included in this review are International Journal of Pharmaceutics, Nature Biotechnology, International Journal of Nanomedicine, European Journal of Cancer, Journal of Controlled Release, International Journal of Biological Macromolecules, Therapeutic Nanoparticles for Drug Delivery in Cancer, Biochemical Journal, British Journal of Clinical Pharmacology, Journal of Delivery and Targeting of Therapeutic Agents. Cochrane Database of Systematic Reviews was also used for reviewing clinical trials. Thus, this review paper was ideally done with high quality screening from recent advancement of potential and few challenges of Red Blood Cells loaded anti-cancer agents.

Chapter 3

Comparative Analysis Among Drug Carriers

3.1 Drug Carriers for Leukemia

Table 2: Some Important Features of Drug Carriers for Leukemia Treatment

Name of the carrier	Therapeutic Indication in Cancer Treatment	Main Feature	Limitations
Polymer therapeutics such as PEG–protein conjugate, PEG–L-asparaginase(Pérez-Herrero & Fernández-Medarde, 2015)	Children with acute lymphoblastic leukemia	Significantly reduces serum ASN levels and kills tumour cells by denying them of an important component necessary for protein synthesis(Dinndorf et al., 2007).	Antibodies that neutralize the enzyme can diminish their action, resulting in the inability of the drug to deplete asparagine following re administration(Dinndorf et al., 2007).
Gold nanoparticles	Acute myeloid leukaemia	Gold nanoparticles penetrated the cell via (apparently) endocytosis, exhibited no toxicity, and significantly lowered reactive oxygen species	Injecting gold nanoparticles into the bloodstream may result in either clotting or haemolysis. After being absorbed by the reticuloendothelial system, nanomaterials

		levels. The toxicity of gold nanoparticles with a citrate coating (spheres 10 nm in diameter) on dendritic cells (part of the human immune system, which process and present antigens on their surfaces for other cells)(Alkilany & Murphy, 2010)	may accumulate in the liver and spleen, posing hepatic and splenic toxicity(Alkilany & Murphy, 2010; Bourquin et al., 2018)
Folate or folic acid as targeting ligand	chronic and acute myelogenous leukemias	The polar PEG linker was attached to the -carboxylic acid to increase the water solubility of FA and to allow for the establishment of an immunological synapse between cancer cells and T cells(Kularatne et al., 2013).	Due to folate's strong affinity for its receptor, some conjugates may release from the receptor too slowly to allow for therapeutic dose build-up, resulting in binding site barrier difficulties(Low & Antony, 2004).

Vincristine-Loaded and sgc8-Modified Liposome	Acute Lymphoblastic Leukaemia	The polyethylene glycol chains form a steric barrier from around liposome, which is expected to keep it from being cleared by the patient's mononuclear phagocyte system during liposome opsonization and to reduce contact with serum proteins. This prolongs the duration of circulation and may change the liposome's biodistribution, hence increasing tumour-specific liposome aggregation and functional interaction with tumour cells	In vivo, they are predominantly rapidly removed by the reticuloendothelial system (RES) and have a short retention duration in the circulating blood. Additionally, there is a lack of physical and chemical stability, which results in aggregation during storage and an absence of targetable features(Duan et al., 2018).

		(Soosay Raj et al., 2013)	

3.2 Erythrocyte Based Drug Carrier for Leukemia

Anticancer agents such as the antibiotic doxorubicin have been incorporated inside carrier RBCs using a variety of loading strategies, include glutaraldehyde cross-linking of the RBC membrane. This change significantly increases the absorption of RBCs by macrophages and other active phagocytosis cells. RBC encapsulated with the similar anthracycline antibiotic daunorubicin were administered into patients with acute leukaemia and demonstrated a greater duration of drug concentration in plasma and fewer side effects than free drug injection(Muzykantov, 2010). When doxorubicin is administered in combination with NPs, the half-life of doxorubicin is prolonged in the bloodstream. Complement activation, which results in inflammation and adverse effects, can function as an impediment. Thus, encapsulating NPs within RBCs acts as a disguise for the immune system. A magnetic field was used as the stimulus in one study: doxorubicin was loaded into red blood cells (RBCs) and chlorinee6 (Ce6) into poly (ethylene glycol) (PEG)-coated iron oxide nanoparticles (NPs); the NPs were then conjugated to the surface of doxorubicin (Dox)-laden erythrocytes. When an electromagnet was introduced, it resulted in site-specific and continual Dox and Ce6 administration to tumour tissue. This carrier-to-carrier strategy (based on RBCs and magnetic nanoparticles) combines the photodynamic therapy of chlorine with the chemotherapeutic impact of Dox, resulting in tumour growth suppression(Guido et al., 2021).

3.3 Drug Carriers for Hepatocellular Carcinoma

Table 3: Some Important Features of Drug Carriers for Hepatocellular Carcinoma Treatment

Name of the carrier	Main Feature	Limitations

Glypican-3 Targeted Human Heavy Chain Antibody	Due to the fact that it contains the CH_2 and CH_3 sections of the human Fc domain, a human heavy-chain antibody, HN3, was produced that is capable of mediating antibody-dependent cell-mediated cytotoxicity (ADCC)(Hanaoka et al., 2015).	Due to the inability to transmit NIR light topically due to liver pigments, NIR light penetration is extremely limited. Thus, in order for NIR-PIT to be efficacious in curing HCC, light probes would need to be inserted into tumours through catheters or needles via the skin following IR700-HN3 injection(Hanaoka et al., 2015).
Chitosan nanoparticles	CNP was able to generate a positive shift in the cell surface potential, indicating that the first stage in the contact between nanoparticles and the cell membrane was surface charge	Chitosan conjugated with triptolide has a limited solubility in water and a high toxicity, limiting its therapeutic application. Additionally, the medicine containing alkyl chitosan is

	neutralisation. The positive charge shift on the surface and the considerable drop in mitochondrial membrane potential indicated that CNP has membrane perturbing activity, as decreased mitochondrial membrane potential (MMP) was associated with mitochondrial membrane loss and destruction of cell membrane integrity(Qi et al., 2007)	associated with clinical toxicity and a high immunogenicity, which can result in the formation of human anti-mouse antibodies(Bonferoni et al., 2020).
Polymeric ultrasound contrast agents	Ultrasound-induced degradation of PLA agents can lead in drug-loaded polymeric fragments with a diameter of less than 400 nm that are capable of escape the tumour's	UCA may have been unable to penetrate into the tumour's core due to significant intertumoral pressure or insufficient vascularization of necrotic regions inside the tumour (Cochran et

	leaky vasculature and collecting within the interstitium(Cochran et al., 2011; Sharma et al., 2022)	al., 2011).
Glycyrrhetinic acid-functionalized mesoporous silica nanoparticles	Free GA may inhibit the cellular absorption of C-6-MSN-GA by interfering with the binding of C-6-MSN-GA to the GA receptor (Jingjing Li & Chen, 2017)	The primary mechanism of toxicity linked with silica has been its surface chemistry (silanol groups), which can interact with membrane components, resulting in cell lysis and subsequent leakage of cellular components (Jafari et al., 2019).

3.4 Erythrocyte Based Drug Carrier for Hepatocellular Carcinoma

Hepatocellular carcinomas are a very common kind of cancer. Erythrocytes have been used successfully to deliver anticancer agents such as methotrexate, bleomycin, asparginase, and adriamycin. When agents including such daunorubicin are loaded, they rapidly diffuse out of the cells, posing a challenge. This issue can be solved by covalently attaching daunorubicin to the erythrocytic membrane with the aid of gluteraldehyde or cis aconitic acid as a linker (Sah et al., 2011). Another study demonstrated the superiority of the erythrocytic version of methotrexate (MTX-RBC) for intravenous delivery over the free form. The authors used

electroporation to encapsulate the medication (i.e., creating pores in the RBC membrane with an electrical impulse), allowing methotrexate (MTX) to enter the cell. When mice were given this form of the drug over a 10-minute period, almost all of the methotrexate administered in RBCs (0.75–1.0 doses) accumulated in their livers, whereas in control experiments (with the addition of the free form of methotrexate), only 0.25–0.3 of a administered drug accumulated(Koleva et al., 2020).

3.5 Drug Carriers for Breast Cancer

Table 4: Some Important Features of Drug Carriers for Breast Cancer Treatment

Name of the carrier	Main Feature	Limitations
Maurocalcine	CPPs (Cell Penetrating Peptides) are cell penetration peptides that, according to their basic features, prefer to collect within cells. Additionally, they contain DNA binding properties (38) that may operate synergistically with DNA targeting (Aroui et al., 2009).	Threshold for Dox-induced toxicity that is less than the threshold for FACS-detected fluorescence. However, the combined data indicate that all these two cell lines are sufficient for studying the effects of Dox–CPP on both cell lines' drug resistance(Aroui et al., 2009).
Carbon nano tube carrier	The receptors are released, the vesicles	Impurities, nonuniform morphology and

	containing the extracellular particle fuse with lysosomes, triggering the release of the drug particle via the action of lysozymes on endosomes, and the free receptors thus formed are recycled to the plasma membrane for conjugation with other ligand conjugated carbon nanotubes (Rastogi et al., 2014).	structure, wide surface area (which promotes protein opsonization), hydrophobicity, insolubility, and CNTs' proclivity to bundle together are all disadvantages(Rastogi et al., 2014).
Polymeric iron oxide nanoparticles	Over a 0.5-hour incubation period, the formulation demonstrated a high absorption by MCF-7 (breast cancer cell line) cells and a low cytotoxicity toward MCF-7 cells. Additionally, an increase in blood	Large surface to volume ratio, rendering them more biologically reactive, because a large surface area in turn provides a huge number of active sites for interaction, which may in turn yield unfavourable responses(Malhotra et

	residence duration was found, as was a significant increase in AUMC(Panda et al., 2019).	al., 2020).

3.6 Erythrocyte Loaded Drug Carriers for Breast Cancer

Graphene oxide nanoparticles were synthesized using ICG as a photosensitizer and DOX as a chemotherapeutic agent, with the shell composed of RBC membrane containing FA as a targeting molecule. These NPs demonstrated outstanding biocompatibility and an exceptional capacity to circumvent RES clearance. To enhance the therapeutic effects of partial thromboplastin timed, the RBC membrane was merged with the MCF-7 breast cancer cell membrane and hybridized membrane-camouflaged melanin nanoparticles were created(Jian Li et al., 2019). When the intracellular methotrexate kinetics of red blood cells were compared to those of other cell types, the MTX kinetics of RBCs were found to be significantly different from those of WBCs. Accumulation of MTX appears to occur much more rapidly in WBCs than in RBCs, and the formation of polyglutamated metabolites appears to be increased as well, as Css for MTXGlu1 was significantly lower in all four WBC cell lines, whereas Css for MTXGlu2–7 was significantly higher in three of the four cell lines, compared to the RBC results. RBC MTX Glun concentrations may offer a measure of cumulative exposure to MTX, similar to the curve of a plasma concentration–time curve, due to the long life of RBCs and the intracellular accumulation of MTX Glun over time. As a result, RBC MTX Glun concentrations may have a predicted association with MTX therapy outcomes(Korell et al., 2014).

Chapter 4

Potential Features of Erythrocyte as a Carrier

4.1 Resealed Erythrocyte

Carrier erythrocytes are generated by differentiating erythrocytes from plasma from the test organism. The cells are ruptured and the medication is captured within the erythrocytes using a variety of physical and chemical procedures; they are then resealed, and the resulting carriers are referred to as "resealed erythrocytes" (Hamidi & Tajerzadeh, 2003; Hirlekar et al., 2008). Resealed erythrocytes are biocompatible, however when autonomous or homologous cells are utilized, implying that no triggered immune response storage is possible. After demonstrating therapeutic efficacy, the chemicals are easily biodegradable without producing harmful byproducts. The resealed cells have the same shape and size as normal cells. It facilitates the drug's movement. Intracellularly, the produced cells are inert and have no interaction with other tissues. REDD makes it simple to isolate, select, and load drugs. The drug can be released and degraded by endogenous enzymes prevented. Various sorts of medications Entrapment are a possibility. Without chemical alteration of the substance to be entrapped, entrapment can occur. It is a form of targeted drug delivery. It is a controlled release formulation for parents. It is based on zero-order drug release kinetics. Due to the drug's prolonged circulation in the systemic circulation, it maintained a prolonged period of release(Sah et al., 2011). Numerous mechanisms of drug release have been postulated. Transport of a certain membrane-associated carrier. Phagocytosis of releasing cells by RES macrophages, subsequent drug buildup in the macrophages' interior, and delayed drug release. After subcutaneous delivery, the drug accumulates in lymph nodes and is released via hemolysis(Ramesh et al., 2016).

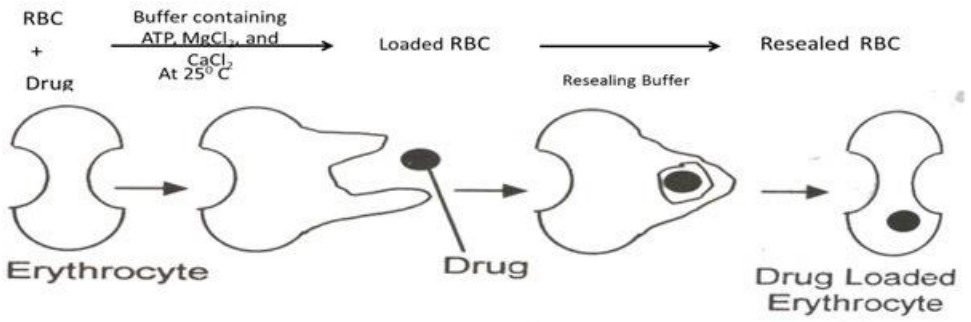

Figure 3: In the erythrocyte entrapment method, resealed erythrocytes are rinsed with buffer solution and sealed with multiple substances entrapped in cells for two minutes at room temperature (Ramesh et al., 2016).

4.2 Nanoerythrosomes

The development of RBC-hitchhiked nanoparticles enhanced their distribution to targeted organs in comparison to lose nanoparticles, which rapidly aggregate in the liver and spleen following injection. To optimize cellular uptake, drug carriers' diameters must be decreased to the nanoscale, which is why erythrocyte membrane-derived nanoerythrosomes (NEs) were established as efficient drug carriers. NEs combine the beneficial qualities of erythrocyte membranes with the increased pharmacokinetics and biodistribution characteristics associated with nanoparticles. Previously, NEs with a mean size of 100–200 nm could be synthesized from erythrocyte ghosts using one of two methods: sonication or extrusion, or a combination of the two(Capossela et al., 2020). Senescent or damaged RBCs are biologically removed by scavenger cells in the spleen, including macrophages and dendritic cells. The spleen is a crucial secondary lymphoid organ that contains an abundance of B cells, T cells, natural killer cells, and antigen-presenting cells (APCs) such as dendritic cells. Damaged RBC could be used to convey tumor-associated antigens to APCs in a crucial secondary lymphoid organ. To do this, nanosized RBC–tumor membrane vesicles or nanoerythrosomes were fused with the membrane of tumor cells using sonication and membrane extrusion to generate antigen–

loaded nanoerythrosomes. Antigen-loaded nanoerythrosomes delivered in vivo effectively reach splenic APCs and trigger T cell immunological responses. In a study using the mouse tumor models B16F10-Luc and 4T1, the combining of antigen–loaded nanoerythrosomes plus an immune checkpoint inhibitor resulted in tumour regression. Additionally, "tailored vaccination" employing antigen–loaded nanoerythrosomes created by fusing RBCs with removed tumors inhibited tumor recurrence and metastasis, simulating a clinical condition(Han et al., 2019).

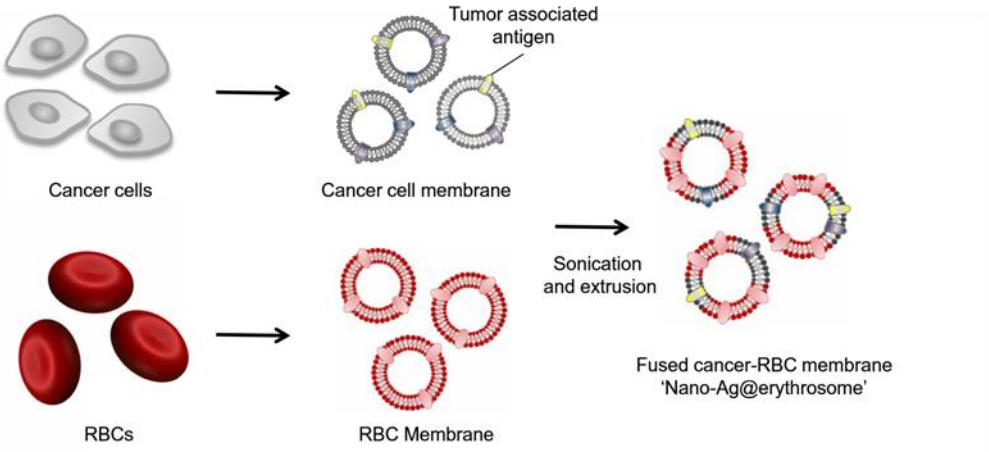

Figure 4: The creation of Antigen-loaded nanoerythrosomes by fusing the membrane of a tumor antigen–associated cell into nanoerythrosomes is depicted schematically(Han et al., 2019).

4.3 Nanoerythrosomes Red Blood Cells as Camouflaged Nanoparticles

Numerous important characteristics of RBCs include their size, morphology, elastic modulus, deformability under circulation, and oxygen-carrying ability. Additionally, we report the introduction of new functionality such as medicinal and diagnostic chemicals into these carriers, enabling them to perform additional functions. Following confirmation of RBC resemblance in vivo by circulation and biocompatibility investigations, the particles described here may provide new avenues for medication administration, medical imaging, and the development of improved disease models (Doshi et al., 2009). The ability to precisely regulate the synthesis process enables the construction of carriers with virtually infinite

properties, functionalities, and geometries (from films to fibers and capsules). The methods for preparing LbL (Layer by Layer) carriers vary and enable the encapsulation of a wide variety of molecules, including antibiotics, growth factors, and biosensor substances, as well as hydrophobic compounds, with the potential of controlled release in intravascular and extravascular target organs (Koleva et al., 2020).

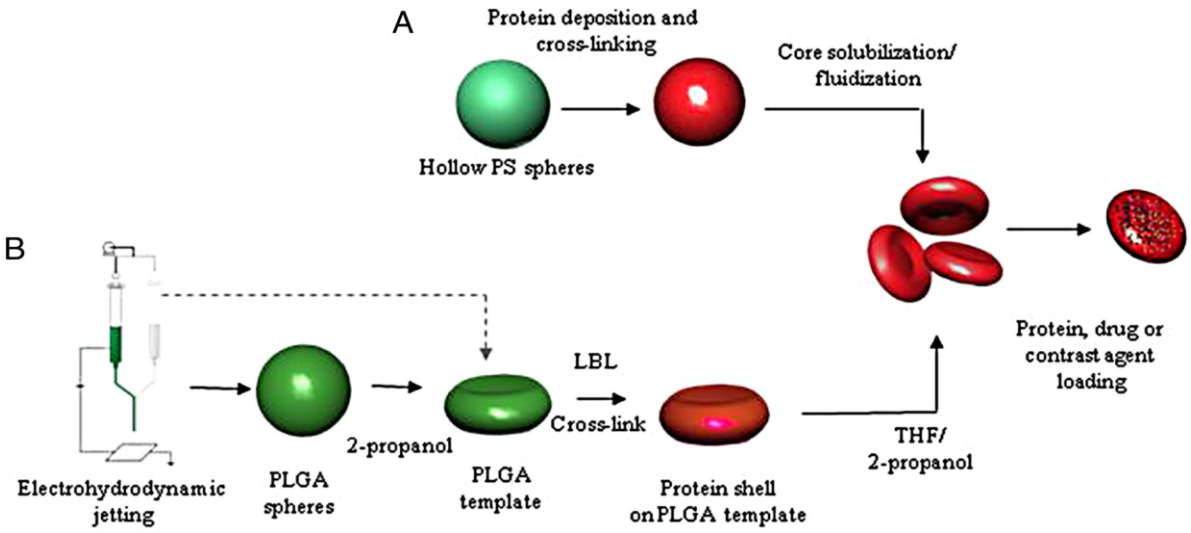

Figure 5: Technique for the synthesis of RBC-like particles. (A) Preparation of RBC-shaped particles using a hollow PS template. On the template surface, complementary layers of proteins and polyelectrolytes were coated using the LbL approach, followed by cross-linking the layers to boost their stability. After dissolving the PS core, RBC-shaped particles were formed that can be loaded with medicinal and imaging substances. (B) Biocompatible RBC-mimicking particles synthesized using a PLGA template. PLGA templates in the shape of RBCs were created by incubating spheres formed by electrohydrodynamic jetting in 2-propanol. LbL coating of the template, protein cross-linking, and template core disintegration resulted in biocompatible sRBCs(Doshi et al., 2009).

4.4 Red Blood Cells as Targeted Carrier in Cancer Immune Therapy

Erythrocytosis' long-lasting circulatory action is mediated by a succession of membrane proteins on the membrane's surface. CD47 is an integral membrane protein having five membrane-spanning domains that firmly embeds it in RBCMs, as well as an IgV-like extracellular domain that promotes to RBCM survival in the circulation. CD47 also functions as a self-marker, signalling macrophages to avoid erythrocyte absorption. Particularly, the signal-regulatory protein alpha (SIRP) glycoprotein expressed by phagocytic cells interacts

with and identifies CD47 as a "do not eat me" signal, preventing immune cells from engulfing RBCs. Other membrane proteins on the surface of RBCMs including such C8 binding protein (C8bp), homologous restricted protein (HRP), decay acceleration factor (DAF), membrane cofactor protein (MCP), complement receptor 1 (CR1), and CD59 (glycoprotein) also contribute to the defence against complement system attacks(Barclay & Van Den Berg, 2014; Xia et al., 2019). Thus, by representing drug nano transporters as "self," these RBCM surface proteins may dampen the immune response and facilitate long-term systemic circulation. In comparison to PEGylated nanoparticles, which have a half-life of 15.8h, RBCM-coated nanomaterials (RBCM-NPs) have a half-life of 39.6h, more than twice as long38. These findings indicate that the RBCM coating increases circulation duration and decreases RBCM-NPs uptake by the reticuloendothelial system (RES), hence boosting the likelihood of nanoparticles entering the tumour via the EPR effect (C. J. Hu et al., 2011; Xia et al., 2019).

4.5 Vascular delivery of Erythrocyte Loaded Drugs

Several procedures, notably hypotonic dialysis and resealing, can be used to encapsulate drugs into isolated RBC. While medications encapsulated in RBCs are isolated from blood, they can nevertheless exert their effects when they or their substrate permeate through the RBC membrane. Unintentional damage to RBC during encapsulation impairs their biocompatibility and has a detrimental effect on drug administration, except when macrophages are targeted. Undamaging technologies for RBC encapsulation enable the development of therapeutically viable drug carriers that significantly increase the duration in circulation, bioavailability, and impact of payloads. Anti-inflammatory medications delivered by RBCs into phagocytic cells and increased bioavailability of detoxifying enzymes inside the circulation are both appealing instances of this method(Yamasaki, 2014). Under normal circumstances, RBCs will be completely contained to the bloodstream, necessitating the use

of intravascular channels for the administration of RBC-carried medicines (e.g., intravenous and intra-arterial). This exclusive localisation in the bloodstream will reduce RBC-associated medication uptake in tissues, potentially eliminating off-target toxicity (Glassman et al., 2020).

Table 5: Comparison Between Ex Vivo and In Vivo Surface Loading

Ex Vivo Surface Loading	**In Vivo Surface Loading**
Covalent conjugation and intracellular loading, which entail modification and reinfusion of RBCs ex vivo.	The loading of medicines in vivo has the potential to significantly lower the risk of harming RBCs, resulting in fast elimination or severe hemolysis-related toxicities.
After ex vivo modification and loading of RBCs, the complete injected dose will be deposited on a minimal fraction of total RBCs in the circulation.	On the other side, in vivo loading enables fine-tuning of the degree of RBC loaded by infusion rate adjustment.

Nevertheless, a very slow IV infusion, lasting minutes to hours, would result in a smaller load on individual RBCs but a more homogenous distribution over the total RBC population, perhaps resulting in fewer deleterious effects on the RBCs and longer circulation durations(Glassman et al., 2020)

4.6 Clinical Trial

In this review, few RBC loaded anti-cancer drugs are specified which have already gone under clinical trial.

4.6.1 Daunorubicin

Daunorubicin was covalently coupled to nanoerythrosomes utilizing glutaraldehyde as the homo bi - functional linking arm, the cytotoxicity of the conjugated drug was shown to be significantly greater than that of the free drug when tested on the P388D1 cancer cell line(Javed, Alshehri, Shoaib, Ahsan, Hadi Sultan, et al., 2021).

The experiment was carried out in a randomized controlled trial using a two-by-two factorial design. Therapy consisted of DA vs ADE, ATRA (all-trans retinoic acid) vs no ATRA, and allogeneic stem cell transplantation with or without Azacytidine. The median duration of follow-up was 18.7 months. The participants met the eligibility criteria for De novo high-risk (acute myeloid leukemia) AML and Secondary AML. Patients beyond the age of 60 were deemed unfit for the MRC AML 15 study due to their high risk. Additionally, individuals who have undergone cytotoxic chemotherapy for AML in the past, who have concurrent active malignancy, who are pregnant or nursing, and who have impaired liver function were excluded. Participants were randomly assigned (N = 616) and ranged in age from 53 to 82 years, with a mean age of 67(Küley-BagheriY, 2018).

The result showed that 50 mg/m2 days 1–3 + 100 mg/m2 cytarabine days 1–10 or days 1–8 and ADE was found to be DA+ etoposide 100 mg/m2/day on days 1–5 of courses 1+2. Overall survival, full recovery, duration of recovery, relapse rates, and mortality in first complete remission were primary outcomes, while toxicity was a secondary outcome. (Küley-BagheriY, 2018).

4.6.2 Doxorubicin

For improved cancer therapy, a systemically biological drug delivery system utilising doxorubicin-loaded RBC was developed. This resulted in increased in vitro cytotoxicity

against a variety of cancer cell types and higher anticancer efficacy in vivo (S. H. Wu et al., 2021).

The study analysed all randomized control trials (RCTs) that investigated the sequence of anthracyclines and taxanes administration in patients with early breast cancer undergoing adjuvant or neoadjuvant treatment. The trial enrolled women who were at least 18 years old and had early breast cancer that was amenable to adjuvant or neoadjuvant treatment. Prior to taxane chemotherapy, anthracycline-based chemotherapy (doxorubicin, epirubicin, or liposomal doxorubicin) was used. The intervention arm received the similar regimen of drugs as the control arm, but in reverse order. For three or four cycles, doxorubicin was administered intravenously at every dose every 14 or 21 days; liposomal doxorubicin was administered at any dose or frequency.

The primary outcome was overall survival, and it was defined as the time interval from randomisation/study admission and death from any cause. Time from surgery to the first relapse of breast cancer during any site, defined as the growth of different ipsilateral (similar breast as earlier breast cancer) and contralateral (other breast than prior breast cancer) breast cancer. Additionally, there was no evidence of invasive or in situ cancer inside the breast and axillary lymph nodes, and no evidence of invasive carcinoma in the breast (Zaheed et al., 2019).

4.6.3 Methotrexate

The innovative nanoerythrosomes technology can be utilized to treat and prevent cancer metastasis, specifically breast cancer metastasis, by suppressing angiogenesis and trapping circulating endothelial progenitors drawn to the tumour.(Javed, Alshehri, Shoaib, Ahsan, Sultan, et al., 2021).

The method was not described, although it was stratified by tumour diameter, age, and lymph node. The study included 206 women with breast cancer (pT1-2N0-1M0), who had undergone quadrantectomy and axillary dissection, had negative margins, had not previously received radiotherapy (RT), and were between the ages of 18 and 76 years. Interventions The experiment used contemporaneous (cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) synchronized with RT) and sequential (cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) synchronous with RT) chemotherapy (CMF then RT at 7 months). Cyclophosphamide 600 mg/m2 was administered intravenously on days 1, 8, and every 28 days for six cycles.

Breast recurrence-free period was the primary result, followed by survival rates, loco - regional relapse, distant metastases, and toxicity(Hickey et al., 2013).

4.6.4 L-asparaginase

GRASPA is indicated to treat of acute lymphoblastic Leukemia (ALL) in children and adults who have relapsed or are resistant to E. coli asparaginase(National Institute for Health Research (NIHR) Horizon Scanning Centre, 2015).

A prior European phase I/II clinical research was done in 24 patients with relapsed ALL to establish the best amount of homologous RBC encapsulated native E. coli Asp (GRASPA®). GRASPA® was compared to free native Asp in terms of activity and safety at three doses (50, 100, and 150 IU/kg) in combination with conventional chemotherapy. Additionally, the worldwide safety profile has been enhanced by lowering the risk of hypersensitivity, liver toxicity, and coagulation issues. The study was interventional (Clinical Trial) in nature and involved 14 individuals. This was a phase I research evaluating the combination of L-asparaginase encapsulated in red blood cells (Eryaspase) and the CALGB regimen in adult patients with acute lymphoblastic leukaemia and lymphoma. The dose was titrated at 50, 100,

150, or 200 IU/kg and the medicine was L-asparaginase encapsulated in RBC at 50, 100, 150, or 200 IU/kg IV. The study was open to participants aged 18 years and older (Adult, Older Adult), and all sexes were eligible. No healthy volunteers were accepted.

A single dose of GRASPA® 150 IU/kg resulted in an 18.6-day depletion of plasmatic asparagine, comparable to that obtained with eight injections of 10,000 IU/m2 free native asparagine. GRASPA was found to reduce the number and severity of allergic responses and coagulation problems (U.S National Library of Medicine, 2018).

4.6.5 5-fluorouracil

5-fluorouracil (5-FU) has been used to treat a variety of cancers for the last 70 years, including hepatic, colorectal, neck, head, and breast cancers. Due to protein denaturation, surface-coating NPs with a biological cell via surface chemistry presents a significant hurdle. Motivated by the concept of fusing synthetic and natural materials and the requirement for functionalized nanoparticles, a NEs-camouflaged nanoparticle platform was designed. By extruding 5-FU-C-LPs and 5-FU-C-NPs through prepared erythrocyte membranes, coated-NPs with bilayer NEs coated with lipids and surface proteins were created. 5-FU is associated with a number of undesirable side effects, including cardiotoxicity, gastrointestinal problems, thrombocytopenia, neutropenia, and dermatology issues. As a result, it is critical to design a novel drug delivery system capable of increasing the treatment effectiveness of 5-FU while minimizing side effects (AlQahtani et al., 2021).

Randomized techniques were used, and four patients (two in each group) died during the first 30 days following surgery were eliminated from the intention-to-treat analysis. 40 participants with hepatocellular carcinoma with a tumor mass of 50% of the hepatic surface, Okuda stage I or II, and no extrahepatic illness were included in the study. The first intervention was surgery, with 20 patients undergoing extended right, right, and left hemi

hepatectomies; the second intervention was adjuvant/neoadjuvant chemotherapy, with 20 patients receiving lipiodol 10 ml, 58 percent urographin 2 ml, mitomycin C 30 mg, farmorubicin 70 mg, leukovorin 100 mg, 5-fluorouracil 750 mg, and gamma- Chemotherapy was resumed 20 days later, followed by five daily immunotherapy sessions administered through arterial administration of lipiodol 3 ml, 58 percent urographin 0.5 ml, proleukin 1 ml, and gamma-interferon 100 mcg to the liver space occupied by the tumour.

Both mean survival times and surviving fractions were provided, which were not standardized to a specified period, as well as the intra-hepatic recurrence fraction, which was not standardised to a defined period. All members of the adjuvant group had a mild fever(Samuel et al., 2009).

Chapter 5

Challenges of Erythrocyte Based Drug Delivery System

Although erythrocyte offers various noticeable potentials among all the drug carriers, no delivery system is free from its limitations and drawbacks. However, the impact can be altered for improved drug delivery.

5.1 Limitations of Erythrocyte Based Drug Delivery

One of the primary obstacles to successful industrialisation is the development of a reproducible loading technique. It is critical to take source cell variability into consideration for this purpose. Indeed, significant changes in erythrocyte resistance to rupture (osmotic fragility) can be detected between samples of erythrocyte starting material (between 2 and 5 g/L), depending on a variety of criteria such as donor age or blood storage temperature. To avoid significant changes in final product quality and to ensure a constant encapsulation rate, specific process variables (ie, erythrocyte flow velocity and buffer osmolality) should be modified in accordance with the osmotic fragility of the primary donor erythrocyte pellet (Bax & Godfrin, 2016). The use of erythrocytes as a medication carrier in humans carries with it the inherent issues associated with blood transfusion. When two blood types are combined, the blood cells may begin to cluster together in the blood arteries, resulting in a potentially catastrophic condition. As a result, it is critical to determine the acceptor's blood type and the kind of erythrocyte carrier in order to eliminate mismatches prior to the delivery of drug-loaded erythrocytes. Another hereditary issue is the likelihood of disease transmission. As a result, it is critical to screen these carriers for the presence of infectious diseases in order to eliminate any potential of contamination (Harisa et al., 2011).

5.2 Drawbacks of Erythrocyte Based Drug Delivery

In some few cases, erythrocytes encapsulated with the medication may boost the generation of unfavourable metabolites. For example, if doxorubicin-encapsulated erythrocytes were administered, doxorubicinol, a hazardous metabolite of doxorubicin, was created in greater quantities(Harisa et al., 2011). Also, osmotic pressure and crosslinking have the potential to damage RBC membranes. Excessive attachment of bioactive agents to the surface of RBCs can also diminish their flexibility and deformability, resulting in an increased risk of complement activation, the unwanted clearance of RBCs by RES, and a reduction in their circulation time in the blood. On human RBCs, the major complement protective proteins are decay accelerating factor and CD59, whereas the low expression of complement receptor 1 CR1 is insufficient to affect lysis., RBC damage can almost certainly result in complications such as vascular dysfunction by bonding RBCs and kidney failure caused by released Hb (Yan et al., 2017). Moreover, T-cell activity and drug-induced antibodies demonstrate the immune system's sensitivity to apparently small changes in RBC surface features. Furthermore, transfusion of mismatched RBCs based on a single amino acid variation in a single membrane protein (e.g., Jk(a) vs. Jk(b) antigens) might result in polyclonal IgG and potentially fatal hemolysis. Despite the great level of variation in RBC surface proteins, even just a minority of transfusion patients generates alloantibodies (Villa et al., 2017).

5.3 Challenges of Targeted Drug Delivery

The insertion of targeting ligands onto the surface of NCs enhances their cellular uptake by target cells while having no effect on the overall tumour localization. In a study, it was validated by developing a mechanistic model for improved understanding and prediction of the complex interactions between molecule size, ligand binding, and tumour targeting. According to their model, active targeting of NCs with diameters less than 50 nm did not affect the tumour localisation of NCs when compared to non-targeted NCs. This is a

significant distinction between actively targeted NCs and small compounds or antibodies(Rosenblum et al., 2018; Schmidt & Wittrup, 2009). Due to the fact that binding ligands to NCs facilitates their absorption by target cells, this method has been used to enhance the functioning of polymeric molecules (macromolecules, e.g., proteins, RNA, and DNA) to their target cells. These compounds are susceptible to enzymatic breakdown and are unable to pass the cell membrane into target cells to reach their active site. Nevertheless, actively targeted NCs that are intended to deliver macromolecules confront additional physiological hurdles as a means to interact with their target cells. One of the most significant obstacles is evading the endocytic route. NCs are driven to subcellular regions following endocytosis by intracellular trafficking pathways, which may have a deleterious effect on the NCs' fate.(Rosenblum et al., 2018; Sahay et al., 2010).

5.4 Economical Impact

Animal research is prohibitively expensive and has a limited throughput. To screen formulation of RBC derivatives and carriers across numerous formulations and situations, one can begin by determining their susceptibility to haemolysis in buffers of variable ionic strength, as osmotic fragility is a well-known consequence of changed RBC membrane physiology. If the modified RBC formulation is as sensitive to reduced osmolality as naive RBC, further challenges such as mechanical resistance, deformability, complement resistance, pH, and oxidative stress can be studied. These are only a few of the numerous stressors that carrier RBCs may undergo throughout circulation(Villa et al., 2016). Sensitive and reliable tests are required to pre-screen several iterations of nanocarriers ahead to costly and time-consuming animal trials. Red blood cells (RBCs), which are contacted by all circulating delivery systems, provide a relatively high-throughput, reproducible, and physiologically relevant model system for assessing carriers' unintended effects. RBCs are perhaps the most readily available biological material from any species, including humans,

which explains the growth of research assessing the adversity of drug delivery methods using very simple readouts of RBC agglutination and haemolysis. In-vitro testing is more morally consistent, cost effective, allows for high-throughput examination of a variety of circumstances, and enables investigations of human RBCs(Pan et al., 2016; Villa et al., 2016).

Chapter 6

Future Aspects & Conclusion

6.1 Future Aspects

Additional research into techniques will hopefully bring the day closer and finally enable RBC drug delivery to thrive that will be unlikely to have harmful outcomes in patients. Rather than using RBC carriers or RBCm-coated nanoparticles, scientists produced RBCm for direct drug encapsulation, dubbed RBCm-derived nanovesicles. Certain approaches, such as cholesterol enrichment, have previously been developed to increase its drug loading efficiency. Recently, a novel approach for isolating RBC-derived exosomes and functionalizing them with magnetic nanoparticles to improve their targeting capacity has been described. The functionalization process consists of four basic steps: serum extraction, exosome isolation with transferrin-bound magnetic nanoparticles, PBS redispersion, and drug loading (Da Sun et al., 2019). Furthermore, It was demonstrated that chemical conjugation resulted in a much more sustained drug release profile than physical encapsulation so the RBC membranes acted as a barrier, inhibiting the outer diffusion of loaded drug molecules (Y. Sun et al., 2017). To counter the immunogenic disadvantage, a study based on RBC-specific production of a model antigen where the non-responsiveness underlying RBC-reactive CD4+ T lymphocytes is expected to be critical in further elucidating the aetiology of autoimmune haemolytic anaemia. It is the further characterisation of the innate immune CD4+ T cell phenotype, which include anergy or t - cell responses that actively repress immunological responses (Hudson et al., 2012). Additionally, this is the first time that a mouse model with RBC-specific production of a clinically relevant human RBC antigen has been characterised in which C3 is involved in antigen clearance as well as possible antigen modulation. Here, the model of haemolytic transfusion reactions may be used to explore

techniques for downregulating antigen and inhibiting incompatible RBC clearance, hence potentially decreasing transfusion risks (Girard-pierce et al., 2016).

6.2 Conclusion

Numerous cell and membrane coating techniques have been developed for the purpose of developing stealth and targeted nanomedicine for cancer treatment. Among these, RBCs exhibit extraordinary drug transport characteristics, making them ideal for encapsulating a variety of bioactive compounds. Due to their inherent benefits of biocompatibility, long life span, and ease of access, erythrocytes are viewed as a viable cell-mediated drug delivery platform. However, surface functionalization with targeted moieties is required to maximize its effectiveness of delivery to malignancy. Thus, tailored RBCs delivery system has evolved from passive to active carriers as a result of significant advancements in the surface modification of RBCs to specifically target cancer types. These natural cells are endowed with unique features that enable them to transport therapeutic drugs, enhance pharmacokinetics, alter pharmacodynamics, and modulate immunological responses. Thus, erythrocyte loaded anti-cancer agent has been welcomed considering its potentials.

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