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ERYTHROCYTE BASED NANOMEDICINE: A NOVEL APPROACH FOR TARGETED DRUG DELIVERY SYSTEM

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ABSTRACT

Recently, drug delivery using biological carriers has emerged as one of the most recent topics of research. Approach of using synthetic nanoparticles (NPs) and erythrocytes combination strategy offers an optimal blend of natural and synthetic materials. This combination strategy could serve as an immune-evasive multifunctional platform. This review summarized significant advances in erythrocytes based nanomedicine targeted drug delivery, and included their fabrication process, their unique properties and applications. Erythrocytes or red blood cells, can act as potential carriers for a wide variety of drugs, including analgesic, anticancer, antibacterial, antiviral, and anti-inflammatory, peptides, enzymes, and other macromolecules. Human red blood cells (RBC), after differentiating from erythroblasts in the bone marrow, are released into the blood and survive in the circulation for approximately 115 days. Erythrocytes having a remarkable bio-compatibility, bio-degradability, and life-span in circulation; makes it a desirable vector for the delivery of the drug as nanomedicine. The erythrocytes based nanomedicine is generally synthesized by either physical or chemical methods by incorporating a wide spectrum of therapeutic agents in the form of nanoparticles. In the current scenario, erythrocyte based nanomedicine holds a great potential of the targeting of drug for therapeutic uses and can also be used for the diagnosis of the human diseases. This review focuses on the most recent advancements in the field of erythrocytes based targeted drug delivery and as an excellent and promising nano platform for the novel targeted drug delivery of various drugs especially antineoplastic and thrombolytic drugs along with their potential as a promising diagnostic tool for the identification of different tumors.

KEYWORD: Erythrocyte, Nanomedicine, Targeted Drug Delivery, immune-evasive.

INTRODUCTION

Erythrocytes are biocompatible, biodegradable, possess very long circulation half-lives and also being the most abundant cell in the human body can be loaded with a variety of chemically and biologically active compounds using various chemical and physical methods. Due this specified qualities it has a potential carrier capabilities for delivery of drugs to the targeted site. The current advances in the drug delivery through cell membrane bounded nanomedicine have gained momentum and site-specific (targeted) delivery of bioactive agents through erythrocyte in different therapies has gained in turns of application. The targeted or site-specific drug delivery is the need of an hour because it provides the best effective ways to improve the therapeutic index (TI) of drug while by-passing the unwanted interaction with drug and non-targeted tissue.[1-5] The targeting process of drug can be approaches by either chemical modification or by appropriate carrier.[6,7] Erythrocytes have been extensively used for their potential applications as drug delivering microspheres.[8-10] Generally, "carrier erythrocytes" are prepared by collecting some blood samples from the organism of interest, separation of erythrocytes from plasma and leukocytes, entrapment of the drug in the erythrocytes, and finally resealing the resulting cellular carriers. After the resealed erythrocytes are achieved, they can be injected into the human body, which serves as targeted or site-specific delivery of the drug.[11-13]

Background/History

Dutch scientist Lee Van Hock described erythrocytes in human blood sample in 1674. About a century later, Howson found that these cells are flat discs rather than the globules described by Van Hock. In the 19th century, Hope Seyler identified hemoglobin and its crucial role in oxygen delivery to different tissues.[14-17]

Gardos in 1953, first experiment for entrapping chemical in erythrocyte were performed, who tried to load the "erythrocyte ghosts" by ATP. In 1959, Marsden and Ostling reported the entrapment of dextrans with

molecular weights of 10 to 250 KD in erythrocyte ghosts.[18-22]

In 1967, the first reports on loading the erythrocyte ghosts by therapeutic agents for delivery purposes were published by Ihler et al. and Zimmerman. The term "carrier erythrocytes" was used for the first time in 1979 to describe the drug-loaded erythrocytes.[23-26] Multiple formulations have emerged to clinical trials. Among these formulations, an exciting example is a targeted polymeric nanoparticle (TNP) containing the chemotherapeutic docetaxel (DTXL) for the treatment of patients with solid tumors.[27]

Basic Features of Erythrocyte

Erythrocytes are biconcave discs shaped cells and with diameter of 7-8 micrometer. They consist of oxygen carrying protein haemoglobin, which is a pigment that gives colour red to blood. Mature erythrocytes in humans are rounded, small and biconcave, as though dumbbell-shaped. Erythrocytes are highly specialized for their O_2-CO_2 transport and blood group antigens and Rh factor carrying function. Matured mammalian erythrocytes are devoid of nucleus for efficient and maximum transport of oxygen, moreover erythrocytes lacks mitochondria and generates ATP anaerobically. A healthy adult male and female has about 5.4 million RBC per microlitre of blood and 4.8 million RBC per microlitre of blood respectively. Erythrocytes live only for 120 days because of the wear and tear that their plasma membrane undergoes as they squeeze through blood capillaries.[28-30] Old erythrocytes are selectively removed generally by spleen through phagocytotic cells which recognize on the basis of senescent cell antigen. Physiologic removal of old and damaged erythrocytes is initiated by the appearance of an aging antigen that marks them for death by initiating the binding of IgG antibody and subsequent removal by phagocytes.[31] The erythrocyte membrane consists of a plasma membrane with basic structure including lipids, proteins, and carbohydrates based on the fluid mosaic model in association with unique structure referred as cytoskeleton, which is a network resulting from the cross-linkage of a number of proteins, mainly spectrin and erythrocyte actin. This structure is necessary for the maintenance of the integrity of erythrocyte upon exposure to high shear rates in circulation.[32-34]

Approaches for Erythrocyte based Nanomedicine

The Erythrocyte based drug-delivery systems have abilities to overcome incompatibilities and to convert an API into a safe and effective drug at the desired site of action. In fact, the active pharmaceutical ingredients (APIs) are poorly soluble, highly toxic, unstable and unavailable at target sites in the body. Several different approaches are available to increase solubility, reduce toxicity, improve stability and deliver the API at selected sites in the body when associated with the membrane of erythrocyte.

It is immediately evident that erythrocytes could be conveniently employed as drug-delivery systems for nanomedicine in different ways as shown in fig. no.1. There are three types of nano biological combined carriers using RBCs can be classified as whole red blood cells which have incorporated drug loaded NPs; NPs coated with RBC membrane; and nano-erythrosomes, which can be considered NPs derived from RBC.[35-39]

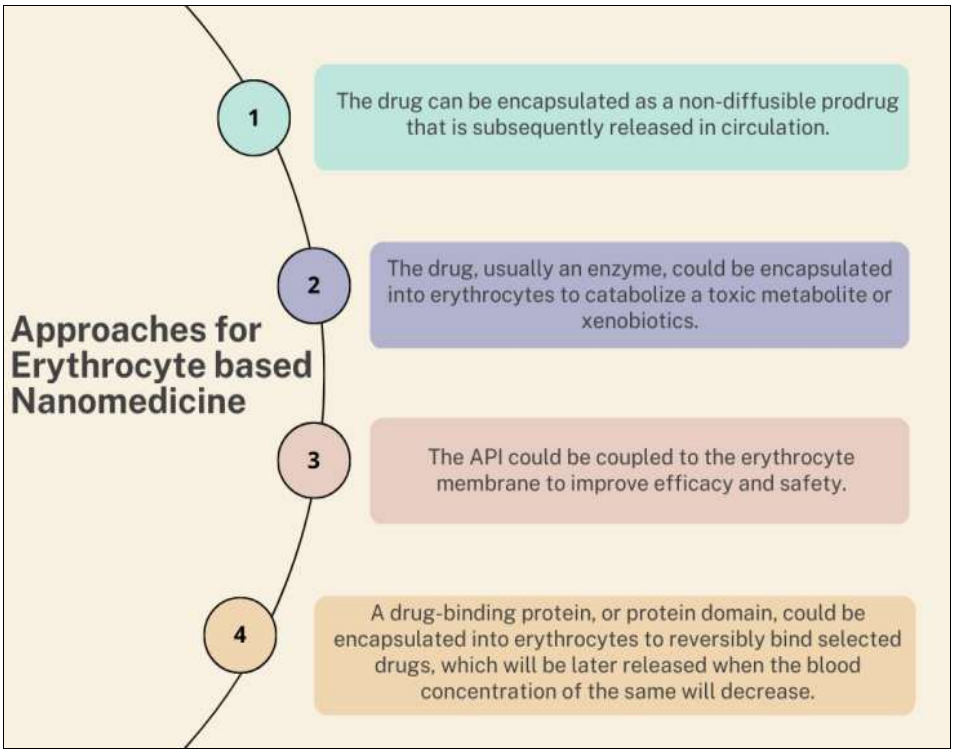

Fig. 1: Approaches for Erythrocyte based Nanomedicine.

Most of the nanoerythrocytes used as drug carriers are rapidly taken up from blood by macrophages of the reticulo- endothelial system (RES), which is present in liver, lung, and spleen of the body. To act as a reservoir for the drug, providing the sustained release of the same into the body, this enables the posology of the drugs to be modified by altering the dose and increasing the dosage intervals. Nanoerythrosomes are prepared by different erythrocyte ghosts to produce small vesicles with an average diameter of 100 nm.

Advantages of Erythrocyte based Nanomedicine

The erythrocytes which are under the process of incorporating the drug or nanomedicine is referred to as encapsulation of erythrocytes. And the outcome of the process is the acquiring of resealed erythrocyte. These have following advantages over conventional drug targeting system:

- A remarkable degree of biocompatibility is the main advantage of this type of cellular carrier.
- These are completely biodegradability and produces minimum toxic products resulting from the carrier biodegradation.
- Give considerable protection of the organism against the toxic effects of the encapsulated drug, e.g. antineoplasts.
- Their life-span is relatively longer in comparison to the synthetic carriers.
- Show desirable size range and considerably uniform size and shape.
- Possibility to achieve targeted drug delivery to the RES organs.
- Maintained relatively inert intracellular environment.
- Possibility to follow zero-order kinetics of drug release.
- Flexibility to entrapped variety of compound within the erythrocytes.
- Show characteristics of high safety, long circulation, high drug loading, and low immunogenicity.
- It helps to modify the pharmacokinetic and pharmacodynamic parameters of the drug.[40-43]

Limitations of Erythrocytes based Nanomedicine

- Carriers can remove in in-vivo study by RES, which limits their usefulness as drug carriers and it may show toxicity in some cases.
- The storage is a further problem occur because these are viable cells and need to survive in circulation for a long time upon re-entry to the host body.
- There may be chances of clumping of cells and dose dumping.
- Several molecules may alter the physiology of the erythrocyte. [44-46]

Methods of drug loading on erythrocytes

Drug-loaded erythrocytes can operate through one of the three main mechanisms of action: prolongation of circulation half-life (bioreactor), sustained drug release, or specific organ targeting delivery. There are different methods for drug loading on erythrocytes as shown in fig. no. 2

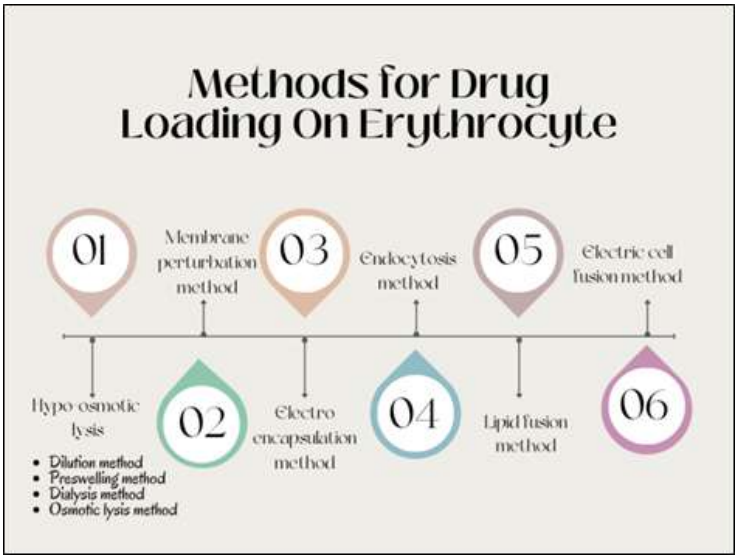

Fig. 2: Methods for drug loading on erythrocytes.

1. Hypo-osmotic lysis method: These are divided into four types are as follows-

- Dilution method
- Pre-swelling method
- Dialysis method
- Osmotic lysis method

(a) Dilution method: According to this method, a volume of packed erythrocytes is diluted with 2–20 volumes of aqueous solution of a drug; the tonicity of solution is then maintained by adding a hypertonic buffer. After that, the resultant mixture is centrifuged, the supernatant is discarded and the pellet is washed with isotonic buffer solution. The

major demerit of this method is its low entrapment efficiency and a considerable loss of hemoglobin and other cell components, which reduces the circulation half-life of the loaded cells. These are phagocytosed by RES macrophages and can be used for targeting RES organs.

(b) Pre-swelling method: It is based upon initial controlled swelling in a hypotonic buffered solution. This mixture is centrifuged at low *g* values. The supernatant is discarded and the cell fraction is brought to the lysis point by adding 100–120 µL portions of an aqueous solution of the drug to be encapsulated. After that the mixture is centrifuged between the drug-addition steps. The tonicity of a cell mixture is restored at the lysis point by adding a calculated amount of hypertonic buffer. The process of preswelling is shown in fig. no. 3. The lysis point is detected by the disappearance of a distinct boundary between the cell fraction and the supernatant upon centrifugation. Lastly the cell suspension is incubated at 37^0C to reanneal the resealed erythrocytes.

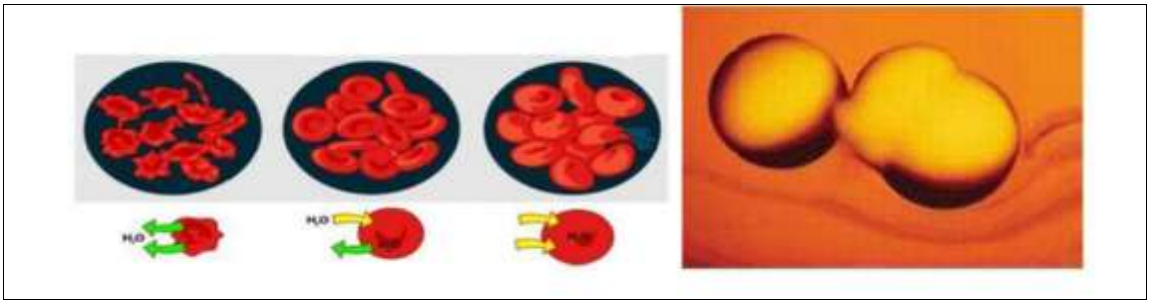

Fig. 3: Hypotonic Preswelling.

(c) Hypotonic dialysis method: This method is based on the principle of semi-permeable dialysis membrane which maximizes the intracellular to extracellular volume ratio for macromolecules during lysis and resealing. A desired hemocrit is achieved in this process by mixing erythrocyte suspension and drug solution. The mixture is placed into dialysis tubing and then both ends of tube are tied with thread. An air bubble of nearly 25% of the internal volume is left in the tube. The tube is placed in the bottle containing 100ml of swelling solution. The bottle is placed at 4^oC for the desired lysis time. The contents of the dialysis tubing are mixed by shaking the tube using the strings. Then dialysis tube is placed in 100 ml of resealing solution. After that the loaded erythrocytes thus obtained carefully washed with cold phosphate buffer at 4°C. the mechanism of the hypotonic dialysis technique is shown in fig. no. 4

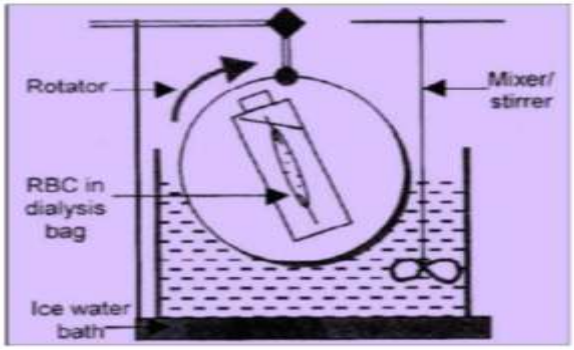

Fig. 4: Hypotonic Dialysis Technique.

(d) Osmotic lysis method: It is also known as the osmotic pulse method. Erythrocytes are incubated in solutions of a substance with high membrane permeability, the solute will diffuse into the cells because of the concentration gradient and it is followed by an influx of water to maintain osmotic equilibrium. Chemicals including urea solution, polyethylene glycol and ammonium chloride have been used for isotonic hemolysis. Finally suspension has been diluted with isotonic-buffered drug solution and cells were separated & resealed at 37^oC.

2. Membrane perturbation method: It is based on the increase in membrane permeability of erythrocytes when the cells are exposed to certain chemicals. These methods have induced irreversible destructive changes in the cell membrane and hence are not very famous.

3. Electro-encapsulation method: It is also known as electroporation method, which is based on using electrolysis leading to generate pores that produce desirable membrane permeability for drug loading into erythrocytes. It involves suspending of erythrocytes in an isotonic buffer in an electrical discharge chamber. It has a capacitor in an external circuit which is charged to a definite voltage and then discharged within a definite time interval through cell suspension to produce a square-wave potential.

4. Entrapment by Endocytosis: Endocytosis involves the addition of one volume of washed packed erythrocytes to nine volumes of buffer containing 2.5 mM ATP, 2.5mM $MgCl_2$ and 1mM $CaCl_2$ followed by incubation for 2 min. at room temperature. The pores created by this method are resealed by using 154 mM of NaCl and incubation at 37°C for 2 min. The entrapment of Endocytosis is shown in fig. no.5. The vesicle membrane separates endocytosed material from cytoplasm & protects it from the erythrocytes.

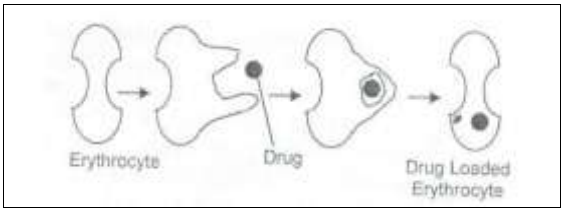

Fig. 5: Entrapment by Endocytosis.

5. Lipid fusion method: Lipid fusion method involves lipid vesicle containing a drug that can be directly fused to human erythrocytes; it leads to an exchange with a lipid-entrapped drug.

6. Electric cell fusion method: It involves initial loading of drug molecules into erythrocytes followed by adhesion of these cells to target cells. The fusion process is promoted by the application of an electric pulse, which causes the release of an entrapped molecule.[47] The process of lipid diffusion method is shown in fig.no.6.

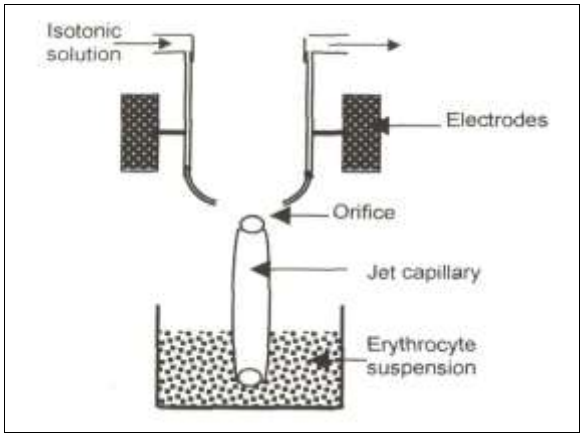

Fig. 6: Liquid Diffusion method.

Application of erythrocytes based nanomedicine

- Erythrocyte-based delivery have been developed to deliver a variety of therapeutic agents, including proteins, nucleic acids, and small molecule drugs.
- Commonly used chemotherapeutic drugs such as vincristine and methotrexate, which are encapsulated in erythrocytes, can improve their efficacy *in-vivo* and prolong the effective time of treatment.
- Erythrocytes cloaked with drug as nanoparticle (nanomedicine) provide a site-specific approach of action to the target tissue or cell.
- Cancer Targeting drug delivery strategies have been developed to improve cancer cell targeting abilities of engineered RBCs and RBC membrane-cloaked nanoparticles.
- In cancer imaging, both engineered RBCs and RBC membrane-cloaked nanoparticles have been applied to improve the image quality of molecular imaging modalities [48]
- Erythrocytes can also be loaded with enzyme, so as to provide effective treatment for enzyme specific site.
- Carrier erythrocyte, encapsulated with native Adenosine deaminase (ADA), has been administered in an adult-type ADA deficient patient demonstrating that encapsulated enzyme is protected from antigenic responses and therapeutic activities are sustained.
- The most successful therapeutic application of enzyme-loaded erythrocytes is represented by the encapsulation of L-asparaginase for the treatment of Acute Lymphoblastic Leukemia.[49]

There are number of disease are related to erythrocytes are shown in Table no.1.

Table 1: Different diseases related to Erythrocytes.

Treatment/ Disease	Name of Drug	Purpose
Lysosomal storage disease	C-glucuronidase, Lysosomal enzymes, 13-galactosidase and 6-glucosidase	It is used to deliver lysosomal enzymes and drugs to lysosomes of the erythrophagocytic cells.
Gaucher's disease	Glucocerebrosidase	By using this loaded cells survived for 10 days in treated patient and mo adverse reactions were found with respect to blood counts, blood pressure and renal functions.
Liver tumors	Anticancer drugs like Bleomycin, Adriamycin, Caboplatin, Gentamycin, etc.	Used to target hepatic carcinomas
Parasitic disease	Immunoglobulin-G coated erythrocytes, Pentamidine loaded	Treatment of parasitic diseases by targeting drug in which the parasite resides in the organs of RES.
Removal of toxic substances	Murine carrier erythrocytes containing bovine rhodanese thiosulphate	Used to antagonize the lethal effects of potassium cyanide in mice or antagonism of cyanide intoxication

Influence of Erythrocyte on Nanomedicine (Nanoparticle) Targeted Delivery

- Particulate nature of blood has been found to influence the targeted delivery of Nanoparticles in the literature. Various models of nanoparticles has been elucidated by[50] which shows how the factors in those models has influenced the targeted delivery.
- Effect of RBC's on the nanoparticles targeted delivery were given by him using the simulation setups like pure particles, RBC's mixed with particles to keep the amount of particles same as that of RBC's and particles mixed with RBC's to keep the concentration constant excluding RBC volume.
- Comparing the results of all the models,[51] found nanoparticles binding rate is higher for the models wherein the RBC's were mixed with particles than the pure particles.
- Adhesion kinetics were influence too due to the mixing of RBC's with particles and shear too had great effect on this kinetic. Furthermore, margination of the particles near the walls of blood vessels has also been found to increase binding rates.

Drug Release

The kinetics of drug efflux from the nanomedicine loaded erythrocytes is an important factor in the plasma concentration profile of the drug upon administration of the encapsulated erythrocytes. Based on the in vitro release experiments on erythrocytes loaded by different drugs, three general release patterns are distinguishable:

1. The rate of drug release is considerably higher than the rate of hemoglobin release (an indicator of the extent of hemolysis). In other words, the drug can diffuse readily out of the intact cells. This type of release pattern has been reported for relatively lipophilic drugs such as phenytoin,[52] primaquin,[53] etc.
2. The rate of drug release is comparable to that of hemoglobin. This means that the drug release is not due to the diffusion out of the intact cells, and instead the drug release occurs only after complete cell lysis. In fact, for this group of drugs, the hemolysis is a prerequisite for the drug release. This type of release kinetics has been reported for the polar drugs such as heparin [54] and gentamicin.[55]
3. The rate of drug release lies between the above-mentioned two extremes. Some drugs such as metronidazole, propranolol, and isoniazid have shown this type of release kinetics.[56]

Potential Future

Erythrocytes loaded nanomedicine have showed many advantages, especially in the concept of excellent biocompatibility and long circulation. For erythrocyte membrane coated nanomedicine, the nano-size structure makes them more feasible to achieve site-specific drug delivery than whole cells as drug carriers. Due to its excellent bio-compatibility, it is also addressed as biomimetics and these biomimetic acts as an elegant method for personalized medicine. The drug delivery nanocarriers are designed to individual patients with little risk of immunogenicity by using their own erythrocytes.

In terms of erythrocyte use for nanomedicine, the current studies are far from sufficient. Biocompatibility of erythrocytes might get altered due to drug loading, and therefore requires a strict control of this aspect for safe and efficacious use of nanomedicine in the course of treatment. The main concern remains is that it requires the use of a patient's own blood cells or blood-type-matched erythrocytes, and thus questions concerning source and storage need to be addressed. Tissue penetration issues of erythrocytes for nanomedicine through microscopic barriers (e.g. the blood–brain barrier, endothelial barriers) have not been well investigated, and the platform should be designed to facilitate accumulation at any site in the body for tumor targeted therapy. In the near future, erythrocyte based nanotechnology will remain an active arena for Nanomedicine.[57]

With the help of multidisciplinary departments like biology, medical science and engineering, the need to develop a more efficient erythrocyte-based nanomedicine platform with high safety and efficacy is necessary.

CONCLUSION

The drug delivery of the nanomedicine through erythrocyte is likely to become more significant in future. Specific, target-oriented drug delivery system are showing promising results along with diminished side-effects and increased therapeutic effects. Both active and passive targeting techniques will enhance the localization of the therapeutic agents in pathological sites. Furthermore, there are various factors regarding the erythrocyte, drug releasing mechanism and other evaluation parameter is being actively investigated for optimization of these systems.

From drug-targeting perspective, they are capable of providing prolong systemic circulation and site-specificity for both passive and active targeting mechanisms, while allowing for the use of synthetic biomaterial such as biocompatible polymers to carry therapeutic agents. For cell-specific targeting, the lipid insertion approach provides these nanoparticles with desirable targeting ligands and controlled density without involving any chemical reactions that may potentially disrupt the protein makeup on the nanoparticle surfaces. Meanwhile, cell membrane-coated nanoparticles carry an antigenic exterior closely mimicking that of the source cells, making them excellent antigen presenting platforms. An effective immune targeting is made possible by tailoring the physicochemical properties of the synthetic cores.

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