Review Article

Erythrocytes: From Oxygen Delivery to Drug Delivery

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Abstract

Few centuries since their first description and several decades after identification of their role in oxygen delivery, now, erythrocytes or red blood cells (RBCs) are among the most widely studied cellular drug carriers. Since the initial burst of interests in this novel drug delivery system more than three decades ago, followed by continuous efforts of several groups worldwide to address intrinsic drawbacks of this promising cellular carrier, and, therefore, the huge body of reports and publications on “Carrier Erythrocytes” or “Resealed Erythrocytes”, they are currently in promising stage of long history and development. In this comprehensive article, the history of erythrocyte and carrier erythrocytes, some biological aspects, methods of preparation, in vitro and in vivo characteristics, delivery strategies, diagnostic and therapeutic applications (including clinical studies), and new generations of carrier erythrocytes is reviewed in details.

Key words: erythrocytes, carrier erythrocytes, drug delivery, reticuloendothelial system, targeted drug delivery

Received: February, 2012 Accepted: April, 2012 Published: April, 2012

1. HISTORICAL BACKGROUND

Erythrocytes, mistaken for fat globules with the early available microscope of the Dutch microscopist, Leeuwenhoek, were first described in the seventeenth century as particles “25,000 times smaller than a fine grain of sand”.1 A more precise description of these cells was given about hundred years later, by Howson finding these cells as flat discs rather than the globules. In the 19th century, Hoppe Seyler completed the Hühnfeld’s discovery of hemoglobin by identification of its crucial role in oxygen delivery to different tissues.2 Reversible oxygenation was considered for a long time as the primary or even sole function of the red cell (along with CO2 exchange) until the late twentieth century. Now, however, our understanding of erythrocyte function has broadened to include O2, CO2, hydrogen sulfide (H2S) and nitric oxide (NO) exchange as well as immune clearance and, possibly, clearance of other soluble blood components such as cytokines.1

The first efforts for entrapment of chemicals in erythrocytes were made in 1953 by Gardos, who tried to load the “erythrocyte ghosts” by ATP.3 In 1959, Marsden and Ostling reported the entrapment of dextrans with molecular weights of 10 to 250 KD in erythrocyte ghosts.4 Fourteen years later, the first reports on loading the erythrocyte ghosts by therapeutic agents for delivery purposes were published independently by Ihler et al.5 and Zimmerman6, and the term “carrier erythrocytes” was used for the first time in 1979 to describe the drug-loaded erythrocytes.7

2. PHYSIOLOGY AND MORPHOLOGY OF ERYTHROCYTES

Erythrocytes, the most abundant cellular constituents of blood (i.e., 5,200,000±300,000 and 4,700,000±300,000 cell/mm³...
blood in healthy men and women, respectively) [8], represent the largest cell specific surface among other blood cells (i.e., the highest surface to volume ratio of 1.9×104 cm/g) [1]. The anuclear mature human erythrocyte is one of the most highly specialized cells. Lacking such cytoplasmic organelles as nucleus, mitochondria, and ribosomes, the red blood cell is unable to synthesize protein, carry out the oxidative reactions associated with mitochondria, or undergo mitosis [1]. Erythrocytes, produced in bone marrow by regulatory effect of erythropoietin [9], making up more than 99% of the total cellular space of blood in humans [10], occupy a volume of approximately 25 to 30 ml/kg, from which 71% constitute an aqueous phase [11]. A total of approximately 760 g of hemoglobin is contained in the erythrocytes, representing approximately 10% of the total body proteins of an adult human10,12-13. In fact, the major function of the erythrocyte is to encase hemoglobin and protect it, so it can act as an oxygen transporter for a prolonged period [14]. Hemoglobin interacts with small diffusible ligands such as O2, CO2, and NO and may be involved in the control of blood pressure [15]. So, thanks to its hemoglobin content, transport of oxygen from lung to tissues and the CO2 produced in tissues back to lung is the main role of the erythrocytes [9]. Erythrocytes draw energy from glucose metabolism via direct glycolysis and the hexose monophosphate shunt [16]. Erythrocytes are flexible biconcave discs with a cell diameter of 7 to 9 µm and a thickness of 2 µm [11,16]. The biconcave disc shape with the highest surface to volume ratio is essential for the gas exchange function of erythrocytes. In addition, this unique shape has a high degree of flexibility required for passage of erythrocytes through the capillaries with diameters of 3-4 µm without undergoing extensive remodeling. The erythrocyte membrane withstands high shear stresses, rapid elongation and folding in the microcirculation and deformation as the erythrocyte passes through the small fenestrations of the spleen [1]. The erythrocyte membrane has a specialized structure consisting of a plasma membrane basic structure including lipids, proteins, and carbohydrates based on the fluid mosaic model in addition to the cytoskeleton. This structure is necessary for the maintenance of the integrity of erythrocyte upon exposure to high shear rates in circulation as well as reticuloendothelial system (RES) [1].

Upon decreasing the osmolarity of the surrounding media, erythrocytes become cup-shaped and finally spherical [1]. This kind of swelling behavior is necessary for the most methods used for loading the erythrocytes by drugs or other chemicals. Erythrocytes have a life span of 100 to 120 days in circulation, during which they travel 250 km throughout the cardiovascular system [16]. As a result of the gradual inactivation of the metabolic pathways of the erythrocyte by ageing, the cell membrane loses its natural integrity, flexibility and chemical composition. These changes, in turn, finally result in the destruction of these cells upon passage through the spleen trabecules [9]. The other effective site for the destruction of the aged or abnormal erythrocytes is the macrophages of the RES including peritoneal macrophages, hepatic Kupffer cells, alveolar macrophages of the lung, peripheral blood monocytes [17], and vascular endothelial cells [18]. It is well known that ageing and a series of other factors make the erythrocytes recognizable by the phagocytizing macrophages via changing the chemical composition of the erythrocyte membrane, i.e., the phospholipid component [17,18].

3. Erythrocytes as Cellular Carriers

At present, there are more than 30 main drug delivery products on the market. The total annual income for all of these is approximately US$33 billion with an annual growth of 15%. The reason for this increasing interest in drug delivery is due to the increasing need of safe and “perfect” drugs, capable of exerting their pharmacological activity only at the target site with minimal side effects on non-target compartments. Novel drug delivery systems, both particulate and non-particulate systems, are modern responses to this urgent need [19]. Cellular carriers, including erythrocytes, leukocytes, platelets, islets, hepatocytes, and fibroblasts are among particulate drug delivery systems that all have been suggested as potential carriers for drugs and biological substances in recent decades. They can be used to provide slow release of entrapped drugs and/or to deliver drugs to specific sites in the body [20]. Erythrocytes, as the most abundant and available cells in the human body have gained the highest degree of interest among the aforementioned cells in recent decades [21] owing to a series of advantages which will be discussed in the next section.

3.1. Advantages and drawbacks

Summarized in Table I, there could be find all advantages and drawbacks of using erythrocytes as particulate drug delivery systems.

3.2. Drug-erythrocyte association

Two major approaches have been in use to associate therapeutic agents and erythrocyte carriers. Carrier erythrocytes can be obtained either through the most widely used encapsulation methods or by reversible [25] or irreversible [26,27] attachment of the drug to erythrocyte membrane.

3.2.1. Drug encapsulation

Almost all methods for the entrapment of chemical and pharmacological agents in erythrocytes take advantage of the remarkable capability of these cells for reversible shape changes and deformation under stress allowing transient opening of pores large enough to be crossed by externally placed molecules [28]. Several methods have been reported for encapsulation of drugs or other bioactive agents in erythrocytes [20], which have either a physical (such as electrical pulse and osmosis-based methods) or a chemical nature (such as the chemical perturbation of the membrane). Whatever the encapsulation method is, a successful drug-entrapment procedure is achieved when the drug to be loaded have the optimal characteristics of considerable degree of water solubility, resistance against inactivation within the erythrocytes, the lack of physical and/or chemical interaction with erythrocyte membrane or other cell constituents, and well-defined pharmacokinetic as well as pharmacodynamic properties [20,21].

All reported methods used for encapsulation of pharmaceuticals and biopharmaceuticals in carrier erythrocytes with a brief explanation of methodologies and some representative...
### Table I. Advantages and drawbacks of carrier erythrocytes [20-24]

<table>
<thead>
<tr>
<th>ADVANTAGES</th>
<th>DRAWBACKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Biocompatibility; particularly in the use of autologous cells;</td>
<td>1. Being biodegradable, they are removed in vivo by the RES. This, although expands its capability to drug targeting, seriously limits their useful life as long-circulating drug carriers and in some cases may pose toxicological problems;</td>
</tr>
<tr>
<td>2. Biodegradability; the lack of toxic biodegradation product(s);</td>
<td>2. The rapid leakage of certain entrapped substances from the loaded erythrocytes;</td>
</tr>
<tr>
<td>3. No undesired immune responses against the entrapped drug;</td>
<td>3. Several molecules may alter the physiology of the erythrocyte;</td>
</tr>
<tr>
<td>4. Considerable protection of the organism against the toxic effects of the entrapped drug, e.g., antineoplasms;</td>
<td>4. Being from biological origin, entrapped erythrocytes may present variability and lesser standardization in their preparation, compared to other carrier systems;</td>
</tr>
<tr>
<td>5. Remarkably longer life-span of the carrier erythrocytes in circulation in comparison to the synthetic particulate carriers and even comparable to normal cells;</td>
<td>5. Inaccessibility of many important therapeutic targets like solid tumors, extravascular tissue components, and central nervous system;</td>
</tr>
<tr>
<td>6. Controllable life-span within a wide range from minutes to months;</td>
<td>6. Safety and technical concerns related to the storage of the loaded erythrocytes;</td>
</tr>
<tr>
<td>7. Desirable size range and the considerably uniform size and shape;</td>
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<tr>
<td>8. Protection of the loaded compound from unwanted degradation within the host body inactivation by the endogenous factors;</td>
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<tr>
<td>9. Possibility of targeted drug delivery to the RES organs;</td>
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<tr>
<td>10. Relatively inert intracellular environment;</td>
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<tr>
<td>11. Availability of the knowledge, techniques, and facilities for handing, transfusion, and work with erythrocytes;</td>
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<tr>
<td>12. Possibility of an ideal zero-order kinetics of drug release;</td>
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<tr>
<td>13. Wide variety of compounds with the capability of being entrapped within the erythrocytes;</td>
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</tr>
<tr>
<td>14. Possibility of loading a relatively high amount of drug in a small volume of erythrocytes, which in turn assures the dose sufficiency in clinical as well as animal studies using a limited volume of erythrocyte samples;</td>
<td></td>
</tr>
<tr>
<td>15. Modification of the pharmacokinetic and pharmacodynamic parameters of the drug;</td>
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<tr>
<td>16. Remarkable decrease in concentration fluctuations in steady state in comparison to the conventional drug-administration methods;</td>
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<tr>
<td>17. Considerable increase in drug dosing intervals with drug concentration in the safe and effective level for a relatively long time;</td>
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<tr>
<td>18. Possibility of decreasing the drug side effects;</td>
<td></td>
</tr>
<tr>
<td>19. Due to their natural roles, they are ideal carriers for intravascular drug delivery;</td>
<td></td>
</tr>
<tr>
<td>20. Possibility of using synthetic erythrocyte counterparts (artificial erythrocytes);</td>
<td></td>
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<tr>
<td>21. Possibility of using special apparatus for loading (i.e. red cell loader) and thus completely controllable loading procedure;</td>
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</tr>
<tr>
<td>22. Possibility of loading without extracting the erythrocytes out the patient body (in vivo loading of circulating erythrocytes).</td>
<td></td>
</tr>
<tr>
<td>Method type</td>
<td>Method name</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Hypotonic hemolysis</td>
<td>1. Separation of RBC</td>
</tr>
<tr>
<td></td>
<td>3. Resealing and reannealing of erythrocyte ghosts</td>
</tr>
<tr>
<td>Hypotonic dilution</td>
<td>1. Separation of RBC</td>
</tr>
<tr>
<td></td>
<td>3. Resealing and reannealing of cells</td>
</tr>
<tr>
<td>Hypotonic dialysis</td>
<td>1. Separation of RBC</td>
</tr>
<tr>
<td></td>
<td>3. Resealing and reannealing of cells</td>
</tr>
<tr>
<td>Hypotonic preswelling</td>
<td>1. Separation of RBC</td>
</tr>
<tr>
<td></td>
<td>3. Resealing and reannealing of cells in hypertonic media</td>
</tr>
<tr>
<td>Osmotic pulse method</td>
<td>1. Separation of RBC</td>
</tr>
<tr>
<td></td>
<td>3. Isotonic dilution with encapsulating agent</td>
</tr>
<tr>
<td>Electrical breakdown or electroporation</td>
<td>1. Separation of RBC</td>
</tr>
</tbody>
</table>
Of the different ways of obtaining carrier erythrocytes, hypotonic dialysis is undoubtedly the most frequently used encapsulation method. This popularity is mainly because of its simplicity, its ease of application for large number of drugs, enzymes, and other substances, and because it is the method that best conserves the morphological and hematological properties of the carrier erythrocytes obtained [29].

3.2.2. Membrane binding

Coupling/binding therapeutics to the surface of carrier erythrocytes represents an alternative to encapsulation strategies that have been considered above. Erythrocyte membrane provides an extended surface area that may be used for anchoring multiple copies of therapeutic molecules. Lack of isolation of a drug from blood en route to the therapeutic site represents an obvious drawback of surface coupling compared with encapsulation. This concern, however, does not seem acute for drugs that are supposed to work in the bloodstream. Furthermore, using pro-drug formulations resistant to plasma inhibitors holds a promise to resolve issues associated with premature inactivation and side effects en route. On the other hand, surface coupling strategies avoid damaging encapsulation procedures exert to the cells and, therefore, offer theoretical advantages of drug loading without compromising erythrocyte biocompatibility. In addition, coupling of drugs to erythrocyte surface circumvents issues related to drug release [84]. Of note, coupling to erythrocyte surface resolves diffusional limitations: even enzymes that react with small, membrane permeable substrate are more active when bound to the erythrocyte surface than when incorporated within the cell [85]. Further, surface coupling offers a unique option to load drugs on circulating erythrocyte without technically and logistically cumbersome need for their extraction necessary for drug encapsulation and re-infusion. Although primary techniques used for erythrocyte membrane binding have been designed in the fifties for lab purposes, however, reliable methods of biocompatible conjugation of drugs to erythrocytes have been designed recently. Several practical strategies for coupling therapeutics to carrier erythrocytes have been evolved and have been tested in vitro and in vivo in last two decades. Generally speaking, these strategies can be divided into three wide categories including: i) chemical coupling of agents to erythrocyte surface (either covalent, or non-covalent); ii) erythrocyte coupling of a receptor that binds a therapeutic agent (and, in some cases, augments its functions); and, iii) conjugation of therapeutics or their receptors with affinity ligands (e.g., antibodies or their fragments) that bind to erythrocyte thereby anchoring cargos on erythrocyte [24].

As the most widely used membrane-binding technology, Avidin-biotin technology has reached several applications in biological sciences [86-88], including drug delivery [89,90], during the two last decades. Membrane association of pharmaceutics, especially biopharmaceuticals, by means of avidin-biotin bridges is the most widely used strategy for non-encapsulation loading of erythrocyte carriers with bioactive

Table II. Erythrocyte encapsulation methods (Contd.)

<table>
<thead>
<tr>
<th>Method</th>
<th>Steps</th>
<th>Example</th>
</tr>
</thead>
</table>
| Laser loading                         | 1. Separation of RBC  
2. Induction of laser pulses on RBCs while in a drug-medium  
3. Resealing and reannealing of cells  
4. Resuspension of cells in blood | N.A     |
| Chemical perturbation of the membrane | 1. Separation of RBC  
2. Agent-induced increasing of RBC membrane permeability (e.g., amphotericin B, urea, ethylene glycol, ammonium chloride, and halothane)  
3. Resealing and reannealing of cells  
4. Resuspension of cells in blood | Daunomycin [86, 75] |
| Drug-induced endocytosis              | 1. Separation of RBC  
2. Exposure of RBCs to membrane-active drugs  
3. Resuspension of cells in blood | Hydrocortisone, propranolol, vitamin A [34], Primaquine, vinblastine, chlorpromazine [76,77,78] |
| Lipid fusion                          | 1. Separation of RBC  
2. Preparation of drug solution in lipidic vesicles  
3. Induction of fusion using a fusogen agent  
4. Separation of fused cells  
5. Resuspension of cells in blood | Inositol monophosphate [79] |
| Intrinsic uptake by erythrocytes      | 1. Separation of RBC  
2. Suspension of cells in drug solution  
3. Incubation  
4. Resuspension of cells in blood | Vitamin B12 [80], Zinc [81-83] |
agents [25,91]. Biotinylation of intact mammalian erythrocytes could be performed either by attachment to the amino groups by means of biotin N-hydroxysuccinimide ester (NHS-biotin) or by oxidation of the induced aldehyde groups of the erythrocyte membrane by biotin hydrazide. Comparison of these different procedures by Magnani and coworkers [91] showed that biotinylation by NHS-biotin provides the highest cell recovery (> 90%); the binding of approximately 1000 biotin molecules per cell (mouse RBC) was achieved with the 24 h survival in circulation being unaffected. Avidin-biotin bridges have been used for reversible membrane binding of uricase,92 HIV-1 Tat protein [93,94], bovine serum albumin (BSA) [25] among several drugs.

3.3. In vitro characteristics

Physical (e.g., morphology, size, loading parameters, surface properties, etc.), cellular (e.g., hemoglobin content, cell recovery, osmotic and turbulence fragility, etc) and biological (e.g., sterility and pyrogenicity) characteristics of carrier erythrocytes can potentially influence the in vivo drug delivery efficiency of these cellular carriers. Therefore, evaluation of these properties in vitro is an important part of the studies on these carriers. A summary of the most widely used in vitro characterization methods is presented in Table III.

3.4. Life span

One of the most important factors influencing the in vivo drug delivering efficacy of the carrier erythrocytes is the survival time of these carriers in circulation upon re-injection. When approaching long circulating delivery and prolonged drug release in circulation, the longer life-span is desired while, if the goal of drug encapsulation in erythrocytes is targeted drug delivery (to the RES or other organs), the rapid removal of carrier cells (shorter lifetime) is preferred. Size, shape, surface electrical charge, and the extent of hemoglobin- and other cell constituents-loss during the loading procedure influence the life-span of the carrier erythrocytes in circulation [20]. Different methods to determine the carrier erythrocytes’ lifetime in circulation include radio-labeling the carrier cells by Cr51 or some fluorescence markers such as fluorescein isothiocyanate (FITC) and encapsulation of C14-containing sucrose or gentamicin [20]. The last two compounds remain within the erythrocytes during its entire lifetime and, consequently, their extracellular concentration in plasma can be used as a measure of the carrier cell disappearance pattern.

A bi-exponential disappearance kinetic of carrier erythrocytes can be seen in circulation starting with a rapid initial decline phase up to 24 hrs after injection (including 15 to 65% of the total injected cells) completing with a slow decline phase with a half-life in the order of days to weeks depending on the animal species used [20,42,44,96,97,98]. The initial rapid disappearance is a result of a considerable number of erythrocytes with abnormal sizes and shapes among the carrier erythrocytes population which were not able of re-gaining their normal morphology during the restoration period of the loading procedure [20,35].

Among different encapsulation methods, the life-span of the white ghosts achieved from the hypotonic dilution method is considerably shorter in comparison to normal erythrocytes [20,35]. The carrier cells produced by the hypotonic dialysis [98], hypotonic preswelling [20,55,99] and electrical breakdown [20] have shown some normal survival times in circulation.

3.4.2 Tissue distribution

Due to natural transporting features of erythrocytes, they may be provide optimal type of carrier for drugs that are either needed to be delivered into erythrocyte-eliminating cells such as RES macrophages, or intended to work in the bloodstream (vascular delivery). When delivery strategy is RES or non-RES targeting, the biodistribution of loaded erythrocytes becomes a critical measure of the system applicability. A series of studies have evaluated the tissue distribution pattern of the drugs encapsulated in erythrocytes; all reporting significant, sometimes dramatic, changes in distribution patterns in favor of the RES organs, mainly liver and spleen, compared to the free equi-dose receiving arms [20].

3.5. Delivery strategies

There are three major strategies in delivery of drugs using erythrocytes as carriers, including intravenous slow drug release, spatially controlled vascular delivery, and targeted drug delivery.

3.5.1. Intravenous slow drug release

The normal life-span of an erythrocyte in systemic circulation is about 120 days [9]. As mentioned as an advantage, in the optimum conditions of the loading procedure (using more gentle methods for loading), the life-span of the resulting carrier cells may be comparable to that of the normal erythrocytes [20,26,30]. Erythrocytes have been used as circulating intravenous slow-releasing carriers for the delivery of antineoplasms agents [20,26,35,48,98,100-102], antiparasitic drugs [59,61,93], anti-retroviral agents [103-111], vitamins [20], steroids [20,30,55,112-114], antibiotics [20,58,75], and cardiovascular drugs [53,54,99] among the others.

A series of mechanisms have been proposed for drug release in circulation from carrier erythrocytes, including passive diffusion out of the loaded cells into circulation, specialized membrane-associated carriers, phagocytosis of the carrier cells by the monocytes of RES and, then, depletion of the drug into circulation, accumulation of the drug in RES upon lysis of the carrier and slow release from this system into circulation [20,61], accumulation of the carrier erythrocytes in lymphatic nodes following subcutaneous injection of the cells followed by and drug release upon hemolysis in these sites, and, finally, hemolysis in the injection sites (in cases of the injection routes other than intravenous) [51].

3.5.2. Spatially controlled (intra)vascular drug delivery

In view of the natural physiology of erythrocytes in the body, they can be used as intravascular (not extravascular) delivery systems of several classes of therapeutic agents. This strategy has been utilized successfully for vascular delivery of some therapeutic enzymes and plasminogen activators coupled to erythrocyte membrane [24,27]. The main improvement of this method compared to the previously mentioned strategy is that the majority of administered drug would remained anchored to carrier erythrocyte and do not release from carrier; So, the extravascular (side)effects are minimal. In the other word, this strategy is assumed to be a combination of sustained drug
## Table III. In vitro characterization of carrier erythrocytes20,21,24,95

<table>
<thead>
<tr>
<th>Parameter(s)</th>
<th>Parameter subtype(s)</th>
<th>Definition</th>
<th>Commonly used techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loading parameters</td>
<td>Loaded amount</td>
<td>The total amount of drug encapsulated in final carrier erythrocytes</td>
<td>Cell lysis/drug assay</td>
</tr>
<tr>
<td></td>
<td>Entrapment efficiency</td>
<td>The percent ratio of the loaded amount of the drug to the amount added during the entire loading process</td>
<td>Cell lysis/drug assay</td>
</tr>
<tr>
<td></td>
<td>Cell recovery</td>
<td>Percent ratio of the volume of the final loaded cells to initial packed cells</td>
<td>Hematocrit determination</td>
</tr>
<tr>
<td>Hematological indices</td>
<td>MCV</td>
<td>Mean corpuscular volume</td>
<td>Coulter counter</td>
</tr>
<tr>
<td></td>
<td>MCH</td>
<td>Mean corpuscular hemoglobin</td>
<td>Coulter counter</td>
</tr>
<tr>
<td></td>
<td>MCHC</td>
<td>Mean corpuscular hemoglobin content</td>
<td>Coulter counter</td>
</tr>
<tr>
<td>Morphology and surface</td>
<td>NA</td>
<td>Evaluation of any changes in morphology of carrier erythrocytes compared with primary RBCs</td>
<td>Scanning electron microscopy, light microscopy</td>
</tr>
<tr>
<td>properties</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Particle size and size</td>
<td>NA</td>
<td>Studying the effects of loading procedure on size and normal curve distribution of final carriers</td>
<td>Laser-based particle size analysis, light microscopy</td>
</tr>
<tr>
<td>distribution</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drug release</td>
<td>NA</td>
<td>The kinetic behavior of drug efflux from the carrier erythrocytes</td>
<td>Diffusion cell, dialysis, simple incubation</td>
</tr>
<tr>
<td>Deformability</td>
<td>NA</td>
<td>Shape changes of the carrier erythrocytes when passing through the narrow pathways in circulation as well as the RES</td>
<td>Capillary passage method, Polycarbonate filter method, chemical membrane deformation</td>
</tr>
<tr>
<td>Osmotic fragility</td>
<td>NA</td>
<td>The resistance of prepared carriers against the changes in osmotic pressure of the surrounding media</td>
<td>Incubation in solutions with different osmolarities</td>
</tr>
<tr>
<td>Turbulence fragility</td>
<td>NA</td>
<td>The resistance of the drug-loaded RBCs against the hemolysis resulting from the turbulent flow within the circulation</td>
<td>Passage through hypodermic needles, vigorous orbital shaking/hemoglobin assay</td>
</tr>
<tr>
<td>In vitro stability</td>
<td>NA</td>
<td>Evaluating the stability of loaded erythrocytes for in vitro storage</td>
<td>Incubation of final carriers in autologous plasma or isotonic buffer, setting the hematocrit between 0.5-5% at temperatures of 4 and 37 °C</td>
</tr>
</tbody>
</table>
delivery and spatially controlled drug delivery to intravascular targets.

3.5.3. Targeted drug delivery
RES or non-RES “targeting” is another important strategy for using erythrocytes as carriers.

3.5.3.1. RES targeting
It is an eminent fact that, in physiologic conditions, as a consequence of the gradual inactivation of the metabolic pathways of the erythrocyte by aging, the cell membrane loses its natural integrity, flexibility and chemical composition. These changes, in turn, finally result in the destruction of these cells upon passage through the spleen trabecules [9]. The other effective site for the destruction of the aged or abnormal erythrocytes is the macrophages of the RES, including peritoneal macrophages, hepatic Kupffer cells, alveolar macrophages of the lung, peripheral blood monocytes [17], and vascular endothelial cells [18]. It has been known that aging and a series of other factors (e.g., stress during non-gentle loading methods) make the erythrocytes recognizable by the phagocytizing macrophages via changing the chemical composition of the erythrocyte membrane, i.e., the phospholipids component [17,18].

Therefore, a considerable fraction of carrier erythrocytes that have undergone some degrees of structural changes during the loading procedure will be trapped by the RES organs, mainly liver and spleen, within a short period of time after re-injection. Consequently, a part of the loaded drug is depleted rapidly in RES [20,61,100], thereby to circulation. It has been shown that up to a definite limit of cell damage during the encapsulation procedure, the spleen is the preferred site for accumulation of carrier erythrocytes [5]. Beyond this limit, drug localization in liver increases remarkably [20]. It is obvious that the more structural alteration in erythrocyte membrane, using more destructive methods, the more rapid and efficient RES targeting will be achieved. This strategy is appropriate for the treatment of specific diseases including: lysosomal storage disease, hepatic tumors and metastases, RES parasitic diseases, iron over-accumulation in the RES, and hepatic porphyria subsequent to lead intoxication [20,21].

A series of approaches have been evaluated to improve RES targeting using carrier erythrocytes. In one of these methods, the drug-loaded erythrocytes is exposed to membrane stabilizing agents, mainly band 3 cross-linking agents, e.g., glutaraldehyde [20,21], bis(sulphosuccinimidyl) suberate (BS(3)) [20,21,115], and 3,3’-dithiobis(sulphosuccinimidyl propionate) (DTSSP) [20,21,115] which may increase the targeting index of the erythrocytes to RES via decreasing the deformability of these cells.

3.5.3.2. Non-RES targeting
Recently, carrier erythrocytes have been used to target organs outside the RES. A variety of approaches including:
A) Co-encapsulation of paramagnetic particles or photosensitive agents in erythrocytes along with the drug to be targeted [20,29,58,116,117];
B) Application of ultrasound waves [118];
C) Site-specific antibody attachment to the erythrocyte membrane (immunoerythrocytes) [20,24,25];

Among the other reports on drug targeting to non-RES organs, preparing the carrier erythrocytes fused to the thermo-responsive liposomes, and their localization using the external thermal source, intraperitoneal injection of the carrier erythrocytes for drug targeting to the peritoneal macrophages [20], and pretreatment of the erythrocytes loaded by the antineoplasm drugs with the lectin extracted from the wheat to improve the targeting to the neoplastic cells [101] all have been associated with improvements in targeting index of the encapsulated drug.

3.6. Routes of administration
3.6.1. Parenteral
Intravenous injection is the main route of administration for drug-loaded erythrocytes. However, these carriers can be administered via other parenteral routes such as subcutaneous and intraperitoneal [20,21,51,119].

3.6.2. Non-parenteral
Although almost all reported routes of administration of carrier erythrocytes are parenteral however, there are few reports of using non-parenteral routes of administration including intranasal delivery of propranolol as bioadhesive system [120] and buccal administration of insulin [121] both tested in rats.

4.APPLICATIONS OF CARRIER ERYTHROCYTES IN DRUG DELIVERY
To the best of our knowledge, inspired by the first reports on loading erythrocytes by therapeutic agents for delivery purposes in 1973 [5,6], red blood cells have been used as delivery system for numerous therapeutic agents both small molecules and large molecules. Also, they have experienced most of clinical applications among cell-based drug delivery systems [122]. In 1977, Beutler and coworkers employed placental glucocerebrosidase entrapped into erythrocytes for a selective delivery to macrophages for the first time[123]. Adenosine deaminase [124], L-asparaginase [125], desferrioxamine [45], thymidine phosphorylase [126], and dexamethasone [112-114,127-129], are among other drugs entrapped in erythrocytes and tested in human studies. These findings, in particular recent clinical trials and orphan drug designation of dexamethasone-loaded erythrocytes for the treatment of cystic fibrosis (Orphan Drug Designation EMEA/OD/039/04EU/3/04/230), are the basis for great hope that erythrocytes have considerable potentialities in the field of drug delivery. Following, almost all attempts in this field would be reviewed in details.

4.1. Delivery of diagnostic agents
Although the majority of reports on using erythrocytes as carriers are focused on loading of drugs from several therapeutic classes but, there are also few reports and a patent (Patent application number: 20100061937) about the using of these cellular carriers for delivery of diagnostic agents.

In a representative study by Johnson KM et al [130], human and rat erythrocytes were loaded with gadolinium DTPA dimeglumine using osmotic pulse technique to create a blood pool contrast agent for MRI. Conventional magnetic resonance contrast agents have drawbacks when used to study tissue perfusion. Current agents such as gadolinium DTPA dimeglumine pass rapidly out of the intravascular space into...
the extravascular space and can diffuse back into vessels. An agent that remained entirely within the vasculature would permit measurement of regional blood volumes and make possible steady-state experimental preparations with which the influence of exercise, drugs, or other factors on perfusion physiology could be studied. In many respects, native erythrocytes would be an ideal contrast agent, and, in fact, the increase in blood relaxivity caused by red blood cell deoxyhemoglobin is exploited in functional brain imaging [130-132]. Based on this rationale and according to the successful in vitro and in vivo results of these studies, it could be concluded that this new system might be useful as a promising and non-invasive diagnostic tool. Altogether, it has been found that usage of other biological carriers for contrast agents such as human serum albumin also demonstrated comparable result [133].

More recently, superparamagnetic iron oxide nanoparticles (SPIONs) have been loaded into carrier erythrocytes to circumvent the unwanted phagocytosis of these nanoparticles in vivo. In fact, the aim of this work was to extend circulation time of SPIONs in bloodstream so that the resulting contrast agent could be used for outcome control after surgery, to monitor healing processes in blood vessels, and to detect internal bleeding. In this work, the authors have demonstrated that, using hypotonic dialysis method, SPIONs can be loaded into erythrocytes in a concentration sufficient to obtain strong contrast enhancement in magnetic resonance imaging [134]. Same results has been achieved by Antonelli et al. which showed that encapsulation of SPIONs in RBC not only increase their half-life in blood circulation, but preserving the properties of nanoparticles also resulted in better activity [135].

4.2. Delivery of therapeutic agents
Erythrocytes have been used for delivery of so many therapeutic agents from several therapeutic groups, both registered and in development ones. As a simplified approach in this review, all studied drugs will be classified in two main groups named pharmaceuticals and biopharmaceuticals. In addition, there is another level of sub classification for each main group.

4.2.1. Erythrocyte-based delivery of pharmaceuticals
4.2.1.1. Anticancer agents
Generally, loading anticancer drugs into carriers controls their toxicity to the body in addition of improving their delivery to tumors relies via several mechanisms, including both specific (i.e., active targeting for example antibody targeting) and less specific (i.e., passive targeting for example Enhanced Permeation and Retention effect, EPR, typical of solid tumors) [136]. Erythrocytes have been evaluated as carriers of chemotherapeutic agents for targeting the RES for about 30 years [137]. From another point of view, carrier erythrocytes may find a niche in tumor therapy, for instance, by providing formulations with prolonged circulation. In support of this hypothesis, loading a hydrophobic antitumor agent dequalinium into mouse erythrocyte provided much longer half-life in circulation in comparison with PEG-loposomal formulation (5-6 days vs 4 hours) [136]. Therefore, antineoplastic agents constitute the greatest number of studies both in vitro and in vivo using erythrocytes as long circulating and targeted carriers (see Table IV).

4.2.1.2. Anti-infective agents
Carrier erythrocytes have been used for delivery of three main anti-infective groups including antiparasitic [59,61,153,154], antibiotic [58,95,110,119,155-158], antifungal [159], and antituberculosis [160] (in veterinary) agents. All of these agents have been reviewed in Table V.

4.2.1.3. Anti-inflammatory agents
4.2.1.3.1. Corticosteroids
The most developed application of carrier erythrocyte is in prolonged delivery of dexamethasone which has been tested in vivo in rabbits and humans [95]. Dexamethasone is a glucocorticoid analogue. Corticosteroids (glucocorticoids) are powerful, though non-specific, anti-inflammatory agents which have been widely used in a variety of inflammatory disorders. Their clinical use has been limited by a number of adverse effects including: development of moon face, redistribution of fat, muscle wasting, acne, bruising, thinning of the skin, osteoporosis, exacerbation of diabetes mellitus, suppression of growth in children, and cataracts. Systemic adverse effects are related to both dose and duration of treatment. Doses lower than 0.3 mg/day cause no suppression of plasma cortisol. Low doses of corticosteroids constantly delivered may be beneficial and without side effects. Based on these rational Magnani [19] and coworkers have encapsulated into autologous erythrocytes dexamethasone-21-phosphate (Dex-21-P) as a non-diffusible produg. Within erythrocyte, Dex-21-P is slowly dephosphorylated by erythrocyte resident enzymes and slowly released in circulation as dexamethasone. Pharmacokinetic investigations in different groups of patients have shown that a single administration of autologous erythrocytes loaded by 8-15 mg of Dex-21-P can release dexamethasone for at least 1 month maintaining therapeutically relevant concentrations of drug in plasma sufficient to saturate the glucocorticoid receptors by 80-85%. The efficacy and safety of this delivery system have been successfully tested in chronic obstructive pulmonary disease (COPD) patients [112], cystic fibrosis patients [113], and in the inflammatory bowel diseases (IBD) [114,127-129]. Until now more than 2000 treatments have been performed in different clinical centers without relevant side effects, exceptional tolerability and acceptability of the treatments by the patients, and documented efficacy [19]. As mentioned earlier, the European Medicinal Agency (EMEA) has also granted the state of orphan drug designation to this treatment for cystic fibrosis patients (Orphan Drug Designation EMEA/OD/039/04EU/3/04/230) [19]. Erythrocyte, as drug delivery system, has recently been used for encapsulation of another systemic corticosteroid, prednisolone [161]. In this investigation erythrocytes obtained from healthy volunteers were loaded with prednisolone using preswell dilution and dilution technique with two different cross-linking agents, glutaraldehyde and dimethylsulfoxide. Carrier erythrocytes, having acceptable loading parameters showed increased percentage drug content with the addition of cross-linking agents. In vitro drug release followed zero-order kinetics, haemoglobin content was found to be satisfactory and osmotic fragility study indicated that increased drug entrapment efficiency was found at 0.3% w/v concentration of sodium chloride (hypotonic solution). In vivo tissue
Table IV. Anticancer agents loaded in carrier erythrocytes

<table>
<thead>
<tr>
<th>Anticancer agent</th>
<th>Approach</th>
<th>Stage of development</th>
<th>Finding(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin-D</td>
<td>RES-targeting</td>
<td>In vitro</td>
<td>High yield of encapsulation was achieved in human erythrocytes, but, with rapid leakage through a diffusion process dependent on temperature. Co-encapsulation of drug with DNA resulted in retaining cells for longer periods, with up to 50% of the encapsulated drug remaining attached to the membrane erythrocyte.</td>
</tr>
<tr>
<td>Adriamycin (doxorubicin)</td>
<td>RES- and non-RES targeting, long-circulating</td>
<td>In vivo (human)</td>
<td>Drug encapsulation in human erythrocytes has revealed a limited biotransformation of the drug at intracellular level, a slow release of the unmodified molecule to the medium and an absence of significant erythrocyte damage. The encapsulation in erythrocytes would increase its antitumor activity and reduce its side effects, largely cardiotoxicity. The treatment of final carriers with glutaraldehyde produced a considerable decrease in the leakage of the unmodified drug, selective liver/lung uptake of the encapsulated drug, as well as more than two fold increase in therapeutic index compared with free drug. Also, by using an experimental model of canine erythrocytes, the erythrocytes loaded with doxorubicin have been treated with antibodies for their selective vectoring to cytotoxic T lymphocytes. More recently, doxorubicin-loaded erythrocytes were administered to 15 lymphoma patients. The drug peak concentration in blood decreased by 55%, doxorubicin circulated several times longer, and the area under the concentration-time curve increased 5 times compared with free drug. This delivery system was tolerated well by patients.</td>
</tr>
<tr>
<td>Bleomycin</td>
<td>RES-targeting</td>
<td>In vivo</td>
<td>The drug was retained in the red cells without appreciable leakage and it was significantly more effective than the same dose of free drug in suppressing the phagocytic function of the RES in mice.</td>
</tr>
<tr>
<td>Carboplatin</td>
<td>RES-targeting</td>
<td>In vitro</td>
<td>The incubation of the drug-loaded erythrocytes in autologous plasma caused a very slow release of the drug from carriers. The encapsulation of carboplatin in erythrocytes may represent a therapeutic strategy for increasing the drug concentration in target organs, such as the liver.</td>
</tr>
<tr>
<td>Daunomycin</td>
<td>Long-circulating</td>
<td>In vivo (human)</td>
<td>An increase in survival was obtained when the daunomycin-loaded erythrocytes were given to mice bearing L1210 cells. This delivery system may act as a time-release system so that cells are exposed to the drug as they enter DNA synthesis.</td>
</tr>
</tbody>
</table>
Table IV. Anticancer agents loaded in carrier erythrocytes (Contd.)

<table>
<thead>
<tr>
<th>Anticancer Agent</th>
<th>Circulation</th>
<th>Administered</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daunorubicin</td>
<td>Long-circulating</td>
<td>In vivo (human)</td>
<td>In a clinical investigation in leukemic patients, it has been demonstrated that erythrocyte-bound daunorubicin had lesser side effects which made it more tolerable for patients.¹⁴⁶</td>
</tr>
<tr>
<td>Dequalinium</td>
<td>Long-circulating</td>
<td>In vivo</td>
<td>Loading of this hydrophobic antitumor agent into mouse erythrocyte provided much longer half-life in circulation that PEG-liposomal formulation (5-6 days vs 4 hours).¹³⁶</td>
</tr>
<tr>
<td>Etoposide</td>
<td>Long-circulating</td>
<td>In vitro</td>
<td>The encapsulation of etoposide in mouse erythrocytes by hypotonic dialysis with alteration of the membrane with band 3 cross-linkers produced a greater uptake of etoposide by the macrophages mainly by a process of phagocytosis. The toxic effect was greater with encapsulated etoposide than that shown by the free drug.¹⁰²</td>
</tr>
<tr>
<td>5-Fluorouracil</td>
<td>Long-circulating</td>
<td>In vivo</td>
<td>Pharmacokinetic/pharmacodynamic study of 5-Fluorouracil (FU) carrier erythrocyte in mice bearing malignant ascites proved the potential efficacy of RBC-FU by a extra-vascular administration (intraperitoneal).¹⁴⁷</td>
</tr>
<tr>
<td>Methotrexate</td>
<td>RES- and non-RES-targeting, long-circulating</td>
<td>In vivo</td>
<td>The pharmacological efficacy (increase in survival time) of methotrexate-loaded erythrocytes was demonstrated in hepatoma bearing mice.¹⁴⁸ Also, glutaraldehyde-treated cells were rapidly cleared from dog’s circulation by liver and spleen.¹⁴⁹ Recognition of erythrocytes by macrophages in vitro and liver targeting in vivo have been enhanced using biotinylated erythrocytes.¹⁵⁰ Further studies have addressed other alterations to the surface of the erythrocytes loaded with methotrexate using desialation and hemichrome induction by treating with trypsin and Phenylhydrazine.¹⁵¹ A more recently published study has demonstrated that the used hypertonic method for encapsulation of methotrexate in erythrocytes has led to a system with both slow release properties (&gt;3 times longer half life than free drug) as well as liver-targeting characteristics in rats.¹⁵² There is another report about successful co-encapsulation of methotrexate with photosensitizers for targeted cancer therapy.¹¹⁶</td>
</tr>
<tr>
<td>Anti-infective class/agent</td>
<td>Approach</td>
<td>Loading method</td>
<td>Stage of development</td>
</tr>
<tr>
<td>---------------------------</td>
<td>----------</td>
<td>----------------</td>
<td>----------------------</td>
</tr>
<tr>
<td>Antiparasitic agent(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primaquine</td>
<td>RES-targeting</td>
<td>Drug-induced endocytosis</td>
<td>In vitro</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>Long-circulating and RES-targeting</td>
<td>Hypotonic Dialysis</td>
<td>In vitro</td>
</tr>
<tr>
<td>Homidium bromide (a trypanocidal drug)</td>
<td>Long-circulating</td>
<td>Hypotonic dialysis</td>
<td>In vitro</td>
</tr>
<tr>
<td>Antibiotic agent(s)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>RES-targeting</td>
<td>Hypotonic dialysis (followed by opsonization)</td>
<td>in vivo (human test)</td>
</tr>
<tr>
<td>Isoniazide</td>
<td>RES- and non-RES targeting</td>
<td>Hypotonic preswelling</td>
<td>In Vivo</td>
</tr>
<tr>
<td>Ethambutol</td>
<td>RES-targeting</td>
<td>Hypotonic dialysis</td>
<td>In vitro</td>
</tr>
<tr>
<td>Drug</td>
<td>RES-targeting</td>
<td>Hypotonic dialysis</td>
<td>In vivo</td>
</tr>
<tr>
<td>-----------------------</td>
<td>---------------</td>
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<td>------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Amikacin</td>
<td></td>
<td></td>
<td>In vitro results have shown the accumulation of amikacin in peritoneal macrophage cell monolayers indicating that drug-carrier system is phagocytosed by macrophages. In vivo tissue pharmacokinetics of amikacin revealed a greater accumulation of the antibiotic in the spleen, peritoneal macrophages and liver.</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td></td>
<td></td>
<td>The in vitro characterization showed that no changes in cell morphology has been occurred during drug encapsulation, although loaded cell are more fragile and more sensitive to turbulence shock compared to normal cells.</td>
</tr>
</tbody>
</table>

**Antifungal agent(s)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>RES-targeting and long-circulating</th>
<th>Hypotonic hemolysis</th>
<th>In vitro</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amphotericin B</td>
<td></td>
<td></td>
<td>Encapsulation of amphotericin B nanosuspension in carrier erythrocytes has led to appropriate loaded amount and loading efficiency. Upon phagocytosis of carriers, leukocytes showed a slow release of drug over ten days, and no alteration in cell viability; which resulted in an immediate and permanent inhibition of intra- and extracellular fungal activity.</td>
</tr>
</tbody>
</table>

**Antibabesiosis agent(s)**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Long circulating</th>
<th>Hypotonic Dialysis</th>
<th>In vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Imidocarb dipropionate (imizol)</td>
<td></td>
<td></td>
<td>Imizol was encapsulated in murine carrier erythrocytes and injected intraperitoneally in mice, resulting in higher blood levels than in animals that had received the free drug and, finally, in a decrease in parasitemia.</td>
</tr>
</tbody>
</table>
distribution studies were carried out for optimized formulation and order of distribution was found to be Liver>Lung>Kidney>Spleen. The developed drug delivery system is endowed with several exclusive advantages and hence holds potential for further research and clinical application.

4.2.1.3.2. Non-steroidal anti-inflammatory drugs (NSAIDS)

Magnetically responsive carrier erythrocytes have also used for delivery of ibuprofen and diclofenac sodium. The drug-loaded carriers responded effectively for an external magnetic field of 8.0 kOe [117,162].

4.2.1.4. Cardiovascular drugs

Enalaprilat is an angiotensin-converting enzyme (ACE) inhibitor widely used as its esterified orally absorbable prodrug, enalapril, in the management of hypertension and congestive heart failure. Using a hypotonic preswelling method, Hamidi M et al [53]. have shown that human erythrocytes loaded by enalaprilat release the drug in vitro according to zero-order kinetics [54]. The in vivo results in rabbit model have indicated that the area under the ACE inhibition curve versus time over the entire course of study was significantly greater following the administration of the erythrocyte-encapsulated drug compared to the free drug. In addition, encapsulated drug inhibited the serum ACE with a slow trend, more efficiently, over a considerably longer time and in a more reproducible manner, than the free drug, thereby emphasizing the role of carrier erythrocytes as slow-release systemic drug delivery system for this ACE inhibitor [99].

4.2.1.5. Iron chelators

Carrier erythrocytes, encapsulated with desferrioxamin (DF), have been studied extensively for treatment of iron overaccumulation in the thalassemic patients and other forms of anemia that require regular transfusions. As discussed above, the RES is the main site of destruction of old erythrocytes and, consequently, of iron overaccumulation in these patients. A remarkable degree of targeting DF to RES using carrier erythrocytes has been reported [43-45,163].

4.2.1.6. Other conventional drugs

Antioxidant drugs (e.g., copper (II) complexes) [164], vitamins [165], narcotic prodrugs [166], tramadol, and phenytoin (unpublished data) are among other drugs investigated for their delivery using carrier erythrocytes.

4.2.2. Erythrocyte-based delivery of biopharmaceuticals

The term biopharmaceutical is most commonly used to refer to all therapeutic, prophylactic, and in vivo diagnostic agents produced using live organisms or their functional components. At least in the US, biopharmaceuticals are often considered to include products manufactured using both “new” technologies (recombinant DNA and monoclonal antibody/hybridoma) and “old” technologies (fermentation, non-recombinant cell culture-derived proteins, vaccines, and other products from live organisms including blood/plasma products). Thus, a biopharmaceutical results from bio-processing and can, therefore, be defined as the intersection of pharmaceutical technology and biotechnology [21,167]. Therefore, from oversimplified point of view, autologous carrier erythrocytes, as biocarriers and due their origin and their adjuvant-mimicking effects [25,168], could be defined as biopharmaceuticals. These cellular carriers have been extensively used in delivery of new and old biopharmaceuticals which will be reviewed in details below.

4.2.2.1. Therapeutic peptides and proteins

A summary of these amino acid-based biopharmaceuticals (i.e., therapeutic peptides and proteins) that have been delivered using carrier erythrocytes are listed in Table VI.

4.2.2.2. Therapeutic enzymes

Enzymes are widely used as replacement therapy in disease states associated with their deficiency (e.g., Gaucher’s disease, galactosemia and hyperurisemia), for degradation of toxic compounds secondary to some kind of intoxication, and as a drug in treatment of different diseases [56]. Unfortunately, some serious problems discourage the direct injection of the enzymes into blood circulation, including the short plasma half-lives of enzymes, toxicity for some tissues, occurrence of some immunologic and allergic reactions, and the need for multiple injections that in turn has the risk of injection-related problems [5,20,35]. One method studied to overcome these problems is the use of enzyme-loaded erythrocytes that can improve the therapeutic outcome via three different mechanisms [21]:

1. Release of the enzyme into circulation upon hemolysis;
2. As a “circulating bioreactor” to which substrate(s) can enter and after completion of the enzymatic reaction, the product(s) can be released out;
3. Accumulation of the enzyme in RES upon hemolysis and catalysis of the enzymatic reaction within this system.

Various therapeutic enzymes that have been delivered using erythrocytes as carriers will be discussed in the following sections.

4.2.2.2.1. Amyloid β-degrading peptidases

Alzheimer’s disease (AD) is the most prevalent neurodegenerative disorder of the elderly affecting over 5 million individuals. It is generally believed that amyloid β peptides (Aβ) are the key mediators of the disease. These Aβ peptides are generated through a minor metabolic pathway involving the proteolytic processing of the amyloid precursor protein [177,178]. Within the CNS the accumulation of Aβ is dependent upon the relative rates of synthesis and clearance. Therefore, decrease in Aβ synthesis and increase in its clearance are two main approaches for AD management. To lower plasma Aβ level, Liu Y et al [179] have investigated the use of three Aβ-degrading peptidases (nepilysin, insulysin, and metalloprotease-1) coupled to erythrocyte membrane using avidin-biotin bridges. They have shown that these erythrocyte-loaded can peptidases degrade Aβ peptide in mouse plasma. Finally, they concluded that this novel system represent an alternative to passive immunotherapy.

4.2.2.2.2. Adenosine deaminase and pegademase

Adenosine deaminase (ADA) is an important enzyme used for enzyme replacement therapy in the treatment of severe combined immunodeficiency disease (SCID) associated with a deficiency of ADA. Introduced by Bax and coworkers [124], ADA is still in use and part of a validated clinical protocol. A 9-year evaluation of carrier erythrocyte encapsulated ADA therapy in a patient with adult-type ADA deficiency has
Table VI. Peptides and proteins carried by erythrocytes

<table>
<thead>
<tr>
<th>Loaded agent</th>
<th>Approach</th>
<th>Loading method(s)</th>
<th>Stage of development</th>
<th>Main finding(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Peptides</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glutathione</td>
<td>RES-targeting</td>
<td>Hypotonic Dialysis</td>
<td>In vivo</td>
<td>GSH-loaded erythrocytes have proven as promising candidates to target monocyte-macrophages compartment as the main site of GSH action towards reducing proviral DNA load in AIDS. Several in vivo animal experiments, using GSH-loaded erythrocytes, alone or especially in combination with other antiretroviral drugs, have resulted in better macrophage targeting index.105-109,169, 170</td>
</tr>
<tr>
<td>Dermaseptin S4</td>
<td>Non-RES targeting</td>
<td>Intrinsic uptake by erythrocytes</td>
<td>In vitro</td>
<td>This peptide has enough affinity for the erythrocyte plasma membrane to bind to it, but given the opportunity, the peptide will exit the host erythrocyte and transfer to another target cell for which it has a greater affinity. The efficacy of this drug delivery system (affinity driven molecular transfer) for dermaseptin S4 and related derivatives has been proven on Plasmodium falciarum model.171</td>
</tr>
<tr>
<td>Listeriolysin O</td>
<td>RES-targeting</td>
<td>Hypotonic dialysis</td>
<td>In Vitro</td>
<td>Erythrocytes loaded with listeriolysin O are effective against Mycobacterium avium replication within macrophages. Also, the strategy presented could be useful against other Mycobacteria other (e.g., M. tuberculosis and M. leprae) by itself or as part of an multiple therapy.172</td>
</tr>
<tr>
<td>Ubiquitin analogue (K48R-Ub)</td>
<td>RES-targeting</td>
<td>Hypotonic dialysis</td>
<td>In vitro</td>
<td>K48R-Ub loaded erythrocytes modified and directed to macrophages, have significantly inhibited the production of TNF-alpha, whose gene is under NF-kappaB control. The results suggested that ubiquitin analogues are potent suppressors of TNF-α release in macrophages.173</td>
</tr>
<tr>
<td><strong>Proteins</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Insulin</td>
<td>Long-circulating</td>
<td>Hypotonic dialysis, hypotonic hemolysis</td>
<td>In vivo</td>
<td>The in vivo decline of insulin released from the loaded erythrocytes indicated a biexponential kinetics. Although the pharmacokinetic behavior of plasma insulin between normal and diabetic rabbits was similar, the hypoglycemic effect was very different between them. Moreover, the effective period of insulin-loaded erythrocytes was longer than that of free insulin. These results suggest that the insulin-loaded erythrocytes may be useful as a dosage form for treatment of patients with diabetes mellitus. Also, the co-encapsulation of a protease inhibitor, tolbutamide, with insulin in erythrocytes has resulted in an entrapment efficacy of 5%.56 Erythrocyte ghosts have also been used for</td>
</tr>
</tbody>
</table>
Table VI. Peptides and proteins carried by erythrocytes (Contd.)

<table>
<thead>
<tr>
<th>Protein (EPO)</th>
<th>Method of cell engineering</th>
<th>Cell engineering</th>
<th>Route of administration</th>
<th>Effect of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythropoietin (EPO)</td>
<td>Long-circulating</td>
<td>Hypotonic dialysis</td>
<td>In vivo</td>
<td>Higher plasma half-life EPO was achieved after loading in erythrocytes. The encapsulated EPO stimulated the erythropoiesis in polycythaemic mice more efficiently. These results suggest that the erythrocyte-encapsulated EPO may serve as a promising alternative to the administration of free recombinant human EPO in the clinical settings.</td>
</tr>
<tr>
<td>Interleukin-2 (IL-2)</td>
<td>Long-circulating</td>
<td>Electroporation</td>
<td>In vitro</td>
<td>In vitro characterization of rhIL-2-loaded erythrocytes indicated their potential as long-circulating cellular carriers.</td>
</tr>
<tr>
<td>Interleukin-3 (IL-3)</td>
<td>RES-targeting</td>
<td>Hypotonic hemolysis</td>
<td>In vitro</td>
<td>The efficiency of encapsulation in mouse erythrocytes was higher when the erythrocytes were loaded hypotonically as opposed to the isotonic incubation method. The treatment of carrier erythrocytes with band 3 cross-linking agents increased the extent of recognition of carriers by macrophages in vitro.</td>
</tr>
<tr>
<td>Interferon alpha-2b (IFN α-2b)</td>
<td>RES-targeting</td>
<td>Hypotonic preswelling</td>
<td>In vitro</td>
<td>In vitro characterization of prepared carriers has shown desirable loading efficiency (above 20%) and release kinetics. In addition, erythrocyte carriers became more fragile against osmotic pressure and turbulent flow, and the deformability of the cells decreased significantly upon drug loading procedure. These factors are, totally, indicating of the possibility of targeting these carriers to RES in vivo without any additional band 3 cross-linking.</td>
</tr>
<tr>
<td>Soluble urokinase receptor (suPAR) and single chain urokinase plasminogen activator (scuPA)</td>
<td>Long-circulating</td>
<td>Membrane binding</td>
<td>In vivo</td>
<td>Unlike constitutively active plasminogen activators, single chain urokinase plasminogen activator (scuPA) is activated by plasmin proteolysis or binding to its receptor, uPAR. Conjugation of recombinant soluble uPAR (suPAR) to rat RBC as let to the markedly prolonged circulation time of suPAR. In contrast to scuPA, RBC/suPAR loaded with scuPA did not exhibit increased adhesion to endothelium, while efficiently dissolving fibrin clots. This molecular design provides a promising modality to deliver a prodrug for prevention of thrombosis without extravascular side effects.</td>
</tr>
</tbody>
</table>
showed promising results in terms of immunological, metabolic and clinical parameters [180]. Enzyme replacement therapy with pegademase (PEGylated ADA) is available, but its efficacy is reduced by anti-ADA neutralizing antibody formation [181]. A novel therapeutic approach to treating ADA deficiency is enzyme replacement with PEG-ADA encapsulated within erythrocytes to protect it from antigenic responses; this therapeutic approach is thus particularly suitable for patients who have previously formed neutralizing anti-ADA antibodies against PEG-ADA [180]. The studies in humans using pegademase encapsulated in erythrocytes reveal a significant increase in the half-life of this enzyme, whilst maintaining therapeutic blood levels, providing an interesting outlook for the treatment of this enzymatic deficiency without undesirable anti-ADA antibody formation [124,182].

4.2.2.2.3. Alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH)

An important path for the metabolism of alcohol is its oxidation to acetaldehyde by the ADH and subsequently to acetate by means of ALDH. The acetaldehyde only accumulates in toxicologically significant amounts following the ingestion of ethanol. The encapsulation of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in erythrocytes reduces excessively high levels of alcohol and acetaldehyde in blood due to chronic alcoholism or to genetic causes, through the release of these enzymes into the blood circulation following hemolysis [183,184]. It has been observed that following the administration of ALDH encapsulated in erythrocytes, the blood levels of acetaldehyde in mice previously treated with a high dose of ethanol were 35% lower than the level of acetaldehyde in control mice and no significant differences were found in the levels of acetone [185-187]. Another group have encountered similar results with concentrations of alcohol in blood 43% lower than in the control groups and clearance values of ethanol from blood in intoxicated mice treated with ADH+ALDH–encapsulated erythrocytes were higher than the control [188].

4.2.2.2.4. Alcohol oxidase

The enzyme alcohol oxidase has a higher affinity for methanol, thus making the loaded erythrocytes useful cellular bioreactors able to catabolise methanol. The use of erythrocytes loaded with alcohol oxidase significantly reduces the levels of methanol in mice. The encapsulation of alcohol oxidase in erythrocytes might contribute to detoxification following methanol poisoning [187].

4.2.2.2.5. Alglucerase

Gaucher’s disease is a rare hereditary disorder with β-glucocerebrosidase deficiency. This results in the accumulation of the lipid, glucocerebroside, within macrophages that become very enlarged and known as Gaucher’s cells. This disease leads to a progressive haematological, skeletal and neurological dysfunction. Alglucerase is a modified form of β-glucocerebrosidase and is a unique form of replacement therapy in patients with a confirmed diagnosis of Gaucher’s disease. In vitro studies for RES-targeting of this enzyme using carrier erythrocytes have proved that high concentrations of alglucerase in the formulation and an extended dialysis time (in hypo-osmotic dialysis method) favour the encapsulation of the enzyme [189,190].

4.2.2.2.6. Arginase

Another example of the potential application of encapsulated enzymes in enzyme replacement therapy is the encapsulation of arginase for the possible treatment of patients with hyperargininemia. Primary in vitro studies have shown that, after enzyme loading, erythrocyte capacity for producing urea and catabolising arginine can be increased [36]. The results of in vivo studies by the same group have shown that it is possible to change the metabolic function of a genetically defective erythrocyte by incorporating exogenous human enzyme. The injection of arginase loaded erythrocytes into hyperargininemic rats produced a longer response to arginase compared with free enzyme [191].

4.2.2.2.7. Beta-glucocerebrosidase

This enzyme is among the first and the most widely studied enzymes delivered to RES using erythrocytes as carriers. Despite only in vitro studies by its modified form (alglucerase), there are so many in vivo studies with the native form of β-glucocerebrosidase in Gaucher’s disease. Using a RES targeting policy, β-glucocerebrosidase-loaded erythrocytes by means of hypotonic dialysis method have showed significant increase in the degradation of the glucocerebrosides within the macrophages [5,38,123,192].

4.2.2.2.8. Brinase

Brinase is a fibrinolytic enzyme produced by Aspergillus oryzae. Brinase has been encapsulated in vitro in rabbit erythrocytes with promising results for use in thrombolytic therapy since the inclusion of this drug delivery system into clotting blood revealed almost complete lysis of the clot [193].

4.2.2.2.9. Catalase and PEG-catalase

This native (and its PEGylated form) catalyses the conversion of reactive oxygen species (O2-) to provided defense against these dangerous reactive species in deficiency cases. Hamarat Baysal S et al. have designed a long-circulating bioreactor using erythrocytes as carriers with appropriate in vitro characteristics [194].

4.2.2.2.10. Delta-aminolevulinate dehydratase (ALA-D)

In intoxications by lead there is a reduction in the intraerythrocytic concentration of the enzyme delta aminolevulinate dehydratase (ALA-D), which leads to the accumulation of delta amino levulinic acid in tissues, blood and urine, which is known as acute porphyria. The administration of ALA-D encapsulated in erythrocytes in normal mice does not produce any significant changes in the enzyme activity in blood, liver, spleen or kidney. However, the administration of ALA-D loaded erythrocytes in lead intoxicated mice produces an immediate and permanent recovery in erythrocytic enzyme activity. The entrapped ALA-D was stabilized to allow retention in both circulating and phagocytized red cells [195]. Human studies, also, have shown immediate and permanent recovery in erythrocytic enzyme activity in lead intoxicated patients [196].

4.2.2.2.11. Glutamate dehydrogenase

Glutamate dehydrogenase is a mitochondrial enzyme that reversibly catalyses the oxidative desamination of the glutamate
to α-ketoglutarate. It is involved in the breakdown of the amni- no acids. The encapsulation of glutamate dehydrogenase in erythrocytes has reduced the levels of ammonia in mice with induced hyperammonemia. Hyperammonemia is a major dis- order caused by different serious, such as hepatic encephalop- athy and certain neurological complaints. To prevent its toxic effects, levels of ammonia should be kept low, whereby glu- tamate dehydrogenase-encapsulated erythrocytes may constitu- te a potential mean for reducing high levels of ammonia in the body [197].

4.2.2.12. Glutamine synthetase
Ammonia detoxification can be also performed by its incor- poration in glutamine by glutamine synthetase. In this way, it has been found that glutamine synthetase-loaded erythrocytes can reduce the blood level of ammonia by about 50% in addition of retaining activity for at least 48h [198].

4.2.2.13. Hexokinase and glucose oxidase
Hexokinase is an enzyme that catalyses the ATP-dependent conversion of aldo- and keto-hexose sugars to the hexose- 6-phosphate. Glucose oxidase is a specific enzyme for the catalysis of oxidation of B-D-glucose. Catabolising enzymes such as hexokinase and glucose oxidase encapsulated in erythrocytes may be employed as new therapeutic ways of reducing high levels of blood glucose. In vitro studies with enzyme-loaded human erythrocytes have revealed a marked increase in metabolism of glucose in comparison to normal cells. In vivo studies have shown that a single intraperitoneal administration of hexokinase/glucose oxidase encapsulated erythrocytes in diabetic mice was able to regulate blood glucose at physiological levels for 7 days and injections repeated at intervals of 10 days were effective in the regulation of glucose levels for several weeks [199,200].

4.2.2.14. Lactate-catabolising enzymes
Enzymes that metabolise lactate in the presence of oxygen as lactate 2-mono-oxygenase and lactate oxidase were encapsu- lated separately and co-encapsulated in human and murine erythrocytes. The co-encapsulation of both enzymes results in significant rates of lactate metabolism. The results obtained in vitro have suggested that the encapsulation of lactate- catabolising enzymes may be useful in the treatment of hyperlactatemia. In vivo studies to prove the efficacy of these enzyme-loaded erythrocytes in the removal of blood lactate in mice have failed because of the high aerobic capacity and high lactate metabolism of this animal model [201].

4.2.2.15. L-asparaginase
Another example of the most widely studied enzymes encapsu- lated in erythrocytes is L-asparaginase. Reported in vivo studies are clear examples of how the encapsulation in eryth- rocytes can improve the nature of a treatment. This enzyme breaks down the amino acid that is vital for neoplastic cell and, accordingly, has been employed for the treatment of cer- tain neoplasias, mainly acute lymphoblastic leukemia, espe- cially in paediatrics [202]. When it is administered as a free enzyme it presents a very short serum half-life and, furthermore, its use has been linked to major immunological altera- tions, an immune-suppressing activity, intolerance reactions and even toxicity in certain organs and tissues, mainly the liv- er and pancreas [203]. Its encapsulation in erythrocytes may attenuate these drawbacks, considerably reducing the immu- nological reactions and protecting it from plasma proteases, thus extending its half-life. Different studies on animals have led to these conclusions. In studies performed on monkeys, guinea-pigs [204,205], mice [60,206], and dogs [207,208], it was proven that L-asparaginase loaded into erythrocytes was more effective in eliminating plasma strategy compared with the same dose of free L-asparaginase, injected in solution. A clinical study have shown improvement in both pharmacokinetic and pharmacodynamic profiles following the IV strategy of the encapsulated L-asparaginase. The half-life of the remaining enzyme in circulation is very similar to that of the carrier erythrocytes (about 30 days) compared to the 8–24h of the free enzyme [209].

4.2.2.16. Phosphotriesterase
This enzyme (especially its recombinant version) hydrolyses many organophosphorus compounds, including paraaxon, a potent cholinesterase inhibitor. Paraaxon is rapidly hydro- lysed by this enzyme to p-nitrophenol and diethylphosphate. The encapsulation of recombinant phosphotriesterase has demonstrated in vitro its potential use for the treatment of in- toxications by organophosphates. The addition of paraaxon to reaction mixtures containing phosphotriesterase-loaded murine erythrocytes resulted in the rapid hydrolysis of para- axon. Obviously, these results were not seen with sham-en- capsulated erythrocytes [210-212].

4.2.2.17. Rhodanase (thiosulfate: cyanate transferase)
Rhodanase (cyanide sulphurtransferase) is a mitochondrial enzyme that converts cyanide into thiocyanate in the pres- ence of sulphur donor compounds. These antidotes have a short-term effect whereby the encapsulation of this enzyme may provide major advantages by maintaining its action for a prolonged period of time. Although the primary studies have indicated that resealed mouse erythrocytes containing rhodan- asis and sodium thiosulphates can rapidly metabolise cyanide, but the potential of this system is restricted because of the limited availability of thiosulphate, due to its poor permeability through erythrocyte membrane [213].

4.2.2.18. Tissue-plasminogen activator (tPA)
Sealing of damaged blood vessels by mural clots prevents bleeding, while pathological vessel occlusion by intravascular clots (thrombosis) causes tissue ischemia and damage, initiat- ing acute myocardial infarction, ischemic stroke, pulmonary embolism and peripheral vascular disease, among other com- plications. Thrombosis is the leading cause of mortality and disability in the US [214,215]. Thrombosis and embolism are also common and dangerous complications of surgery that are especially difficult to manage due to the risk of acute bleeding at the operative site. Invasive interventions (e.g., angioplasty, carotid endarterectomy) may be complicated by formation of clots embolized to the brain and cause neurological dis- orders. Emergency therapy of thrombosis employs injection of plasminogen activators including tissue type (tPA) and urokinase (uPA) which cleave fibrin clots and thus restore perfusion [216,217]. However, inadequate delivery (blood clearance within <15 min) [218], inactivation by plasma inhibiters and impermeability of occlusive clots [211] restrict the
effectiveness of therapeutic fibrinolysis by plasminogen activators. Within minutes after infusion of mega-doses of fibrinolytics (e.g., ~100 mg of tPA) needed to overcome its inefficiency and achieve fibrinolysis locally, excess drug diffuses into preexisting hemostatic malar clots predisposing to bleeding and into tissues such as the CNS [220], where it may cause cerebral hemorrhage, damage to the blood-brain barrier and brain toxicity [221]. Due to the danger of bleeding and the collateral CNS damage, fibrinolytics cannot be used in over 95% of stroke patients and in the post-operative period other than as an emergent potentially life-saving intervention. A more ideal thromboprophylactic agent would prevent occlusive thrombosis from forming without lysing hemostatic mural clots and causing extravascular toxicity. Attempts to improve tPA delivery and its outcome have not yielded resolutely better results [222-225]. Prophylactic use of tPA in patients at high risk of imminent primary or recurrent thrombosis will reduce the formation of impermeable occlusive clots due to delays in fibrinolysis. However, fibrinolytics are not used for prophylaxis as results of their rapid clearance and unbearable side effects. Recent animal studies, by Muzykantov group, in mice and rat showed that biocompatible coupling to erythrocyte converts plasminogen activators (in particular tPA) from challenging therapeutic agents into efficient and safe agents for intravascular thromboprophylaxis. Hemodynamic factors are favorable, propelling erythrocyte towards the blood mainstream [226-228], restricting drug contact with vascular walls and mural clots. This delivery system would prevent from complement activation, phagocytosis or accelerated clearance of carriers [229], protects tPA from plasma inhibitors [230], while preserving its fibrin affinity and activation by fibrin [231], prolongs tPA circulation [231] and permitting its use as prophylaxis [229].

4.2.2.21. Urease/AlaDH and PEG-urease/PEG-AlaDH combination system
Urease is an enzyme that catalyses the hydrolysis of urea into carbon dioxide and ammonia. Alanyldehydrogenase (AlaDH) catalyses the reversible oxidative deamination of L-alanine to pyruvate and ammonium. Urease/AlaDH [232] and PEG-urease/PEG-AlaDH [233] enzyme systems have been effectively encapsulated in human erythrocytes in vitro. With these delivery systems, urea is broken down into ammonia and bicarbonate. The ammonia released is converted into alanine by alanine aminotransferase (ALT), then into pyruvate under the catalytic action of AlaDH. The results of in vitro studies suggest the potential application of these combination enzyme systems in the treatment of high blood levels of urea in patients with chronic renal failure.

4.2.2.22. Uricase
This enzyme, isolated in mammals, is responsible for the transformation of uric acid into allantoine. Erythrocyte carriers have also been studied for loading of uricase to develop a bioreactor for uric acid degradation. The concentration of the enzyme can be made arbitrarily high and the only rate-limiting step for the uric acid degradation is the rate of substrate entry, so uricase-loaded erythrocytes are potentially capable of removing as much uric acid as the human kidney. By coupling the uricase to the erythrocyte membrane with a biotin–avidin–biotin–enzyme bridge, Magnani et al. have solved the problem of low permeation rate of uric acid into erythrocytes and keep low plasma concentrations of uric acid for several days [234,235].

4.2.2.23. Urokinase
As described in section 4.2.2.3.16, urokinase is a plasminogen activator that facilitates its conversion to plasmin, which promotes thrombolysis. Urokinase constitutes a useful enzyme in the treatment of recently formed thrombotic or embolic states. Erythrocytes have been proposed as carriers of human urokinase, as a possible form of administration in the treatment of patients with thrombosis as an alternative to the use of high doses of urokinase. In vivo pharmacokinetic studies on rabbits administering free urokinase and urokinase-loaded erythrocytes suggest an uptake in the liver and kidney and the formation of urokinase–proteinase inhibitor complexes. After intravenous administration, the area under the curve of the carrier erythrocytes was estimated to be eight times higher than free urokinase. These results confirm the slow-release of the enzyme from loaded erythrocytes and the protection from plasma inhibitors [236].

β-galactosidase, β-glucosidase, malate dehydrogenase, glutathione reductase, fumarase, glyceraldehyde 3-phosphate dehydrogenase, and superoxide dismutase are among other enzymes that have been loaded into erythrocytes successfully [20,21].

4.2.2.3. Antigen delivery for vaccination
The successful development of vaccine delivery system requires consideration of a number of issues including safety, delivery system and appropriate adjuvant. Erythrocyte carriers have general advantages, those listed in Table 1, which, collectively, make them as an excellent candidate for antigen delivery.

Aside from the general advantages of erythrocytes for using them as a suitable drug delivery system, they have three other remarkable potentials for being used as a vaccine delivery system including [21,25,168,237]:

1. They can be used for controlled release of vaccines with an aim to reduce the number of doses for primary immunization or to develop single dose vaccines;

2. They can act as a vehicle to target antigen to antigen-presenting cells (APC), not only macrophages but also dendritic cells (DCs). Depending on the extent of changes occurred in cell physiology and/or morphology during antigen loading procedure, one can prepare erythrocytes loaded by antigens and be capable of serving as controlled antigen release (in the case of minor cell modifications) or antigen targeting (in the case of major cell modifications) vehicles;

3. The intrinsic adjuvancy of loaded erythrocytes due to loading procedure;

Following, several groups of antigens, delivered by means of erythrocyte carriers, will be reviewed with more details.

4.2.2.3.1. Model antigens and adjuvants
The use of adjuvants is the necessary prerequisite for inducing strong immunological responses to biotechnology-derived antigens like proteins and, especially, subunit peptide vaccines [25,238,239]. However, in many cases, these adjuvants cannot be extensively used in human and veterinary vaccination as a
consequence of associated inflammatory reactions or granuloma formation [25,240]. Magnani et al. have reported that model protein antigens (bovine serum albumin, BSA, hog liver uricase, and yeast hexokinase), coupled to autologous erythrocytes by means of a biotin-avidin-biotin bridge, elicit an immunological response in mice comparable to or even higher than those obtained by the use of Freund's adjuvant. Quantities as low as 0.5 micrograms/mouse are high enough to generate these immunological responses. They concluded that the delivery of antigens by autologous erythrocytes is an effective way to avoid the use of adjuvants for producing anti-peptide antibodies and possibly to generate peptide vaccines [25].

Murray AM et al [241]. have reported specific humoral immune responses after immunization of female Balb/c mice with erythrocyte-loaded model antigens. Immunoglobulins (Ig) G1, G2, and G3 were detected in mice after intravenous injection of murine erythrocytes loaded with Keyhole Limpet Haemocyanine, BSA, and bovine adenosine deaminase as model antigens. This study demonstrated that a single administration of antigen-loaded carrier erythrocytes is able to elicit humoral immune responses comparable or superior to those obtained via the adjuvanted subcutaneous vaccination route. The IgG isotype profiles demonstrate that the erythrocyte entrapment of antigens is another mechanism by which the Th responses to antigens maybe modulated.

As a RES-targeted delivery approach, we, also, have developed BSA-loaded erythrocytes using hypotonic preswelling method with acceptable loading efficiency and other in vitro characteristics for RES targeting [168,242].

4.2.2.3.2. Bacterial Toxoids
Erythrocytes may be of interest in the field of vaccines as natural carriers and/or adjuvants for antigens. Several attempts have been made accordingly. Polvani et al. have encapsulated three bacterial toxoids, a mutant of the diphtheria toxin, the tetanus toxoid and a double mutant of the pertussis toxin in murine erythrocytes by a hypotonic dialysis method. Then, a comparative study was performed by in vivo administering multiple intravenous injections of the different toxoids encapsulated in carrier erythrocytes compared to the same dose of toxoids administered in a saline solution. Titters in sera of specific antibodies were tested. Titters of antibodies against diphtheria toxin and tetanus toxoid were higher upon immunization using toxoid-loaded erythrocytes in comparison to free toxoids [243].

4.2.2.3.3. Viral subunit vaccines
As mentioned in section 4.2.2.3, the use of adjuvants is usually mandatory for the delivery of subunit peptide-vaccines [25,238-240] such as viral subunit vaccines. To minimize the use of adjuvants and related risks, erythrocyte carriers are good candidates for the delivery of retroviral subunit vaccines. The immunotherapeutic potential of autologous erythrocyte coupled to the secretory form of herpes simplex virus type1 (HSV-1) glycoprotein B (gB1s) was evaluated by Chiarantini et al. with a mouse model of HIV-1 infection. Mice immunized with erythrocyte coupled to gB1s were protected against lethal and latent HSV-1 infection, and developed an anti-HSV antibody response similar or higher than that elicited by the ten-fold higher amount of the same antigen in Freund's complete adjuvant, suggesting that autologous erythrocyte coupled to gB1s may provide an effective and safe method of immunization against HSV infection [244].

Dendritic cells (DCs) can represent an important target for vaccine development against viral infections. In a study carried out by Corinti et al. it was shown that erythrocytes deliver the HIV-1 Tat protein efficiently to interferon-gamma-treated human DCs for efficient initiation of specific type 1 immune responses in vitro. Tat was conjugated to RBC (RBC-Tat) through avidin-biotin bridges. DCs internalized RBC-Tat efficiently and compared to DCs pulsed with soluble Tat, DCs incubated with RBC-Tat elicited specific CD4+ and CD8+ T-cell responses at much lower antigen doses [93]. In another study, the immunotherapeutic potential of biologically active recombinant HIV-1 Tat protein coupled to autologous erythrocytes, via avidin-biotin bridges, was evaluated in a mouse model in comparison to free protein. Although anti-Tat antibody responses was similar in both groups, only, 2/6 animals immunized with soluble Tat (free protein group) and 6/6 animals immunized with RBC-Tat developed anti-Tat neutralizing antibodies. In addition, at week 28 cytolytic anti-Tat Cytotoxic T Lymphocytes were detected in all animals although they were slightly higher in mice immunized with RBC-Tat. These results indicated that RBC-mediated delivery of HIV-1 Tat, in amounts 250 times lower than soluble Tat, is safe and induces specific CTL responses and neutralizing antibodies [89].

4.2.2.3.4. Cancer Vaccines
It has been shown previously in several tumor systems that C-reactive protein (CRP) is an effective agent for generation of macrophage-mediated tumoricidal activity by means of suitable delivery systems like multilamellar vesicles (MLV). Gau tam et al. have shown that resealed erythrocyte ghosts can act in the same manner. In their study, CRP associated with erythrocyte ghosts inhibited established lung metastases of T24 fibrosarcoma in C57B1/6J mice. This finding, along with their in vitro findings, indicated the potential value of erythrocytes as another delivery system for biological response modifiers for cancer vaccination to prevent metastases [245]. Very recently, Banz A et al [237]. have shown that erythrocytes are efficient antigen carriers to target DCs and induce cytotoxic T-cell responses for cancer immunotherapy. In this experiment, mouse erythrocytes were loaded with ovalbumin (OVA) and injected with poly (I:C) into mice. Phagocytosis of OVA-loaded erythrocytes by macrophages and DCs was demonstrated to induce OVA-specific CD4+ and CD8+ T cell activation. Moreover, CD8+ T cells produced interferon gamma and were able to induce OVA-specific cell lysis. Finally, T-cell response was confirmed to be dependent on the dose amount of the antigen entrapped and this response could be maintained for up to 30 days.

4.2.2.4. Nucleosides, nucleotides and their analogues
Nucleoside and nucleotide-loaded erythrocytes, especially anti-retroviral nucleosides, nucleotides and their analogues, constitutes one of the most promising therapeutic applications of carrier erythrocytes. Because most antiviral drugs are nucleotide or nucleoside analogues, their entrapment and release
through the cell membrane needs careful consideration. Nucleosides are rapidly transported across the membrane whereas nucleotides are not and, thus, exhibit prolonged release profile. In this way, conversion of moieties to purine or pyrimidine bases is required for the release of nucleotides. Following, we will review the applications of carrier erythrocytes in delivery of these drugs.

4.2.2.4.1. Azidothymidine (AZT) and dideoxyinosine (DDI)
It is a well-known fact that the infectivity and replication of immunodeficiency viruses are inhibited by certain analogues of nucleosides following their intracellular transformation into triphosphate derivatives. The monocyte-macrophage system plays a key role in infection by HIV-1. These cells, become infected immediately after exposure to HIV, are relatively resistant to virus attack and constitute an important reservoir for the virus [105]. Both azidothymidine, AZT; an analogue of thymidine, and dideoxyinosine, DDI; a nucleoside analogue, are reverse transcriptase inhibitors and both are prescribed as anti-HIV drugs. One therapeutic strategy has been based on protecting the macrophages against HIV-1 infection using AZT and DDI co-encapsulated in erythrocytes using a murine AIDS model. Mice treated with AZT-DDI-GSH-loaded erythrocytes, presented a pro-viral DNA content in the brain and in macrophages that was significantly lower than in mice treated with combination of AZT and DDI [105].

4.2.2.4.2. Azidothymidine prodrugs
Disseminated infection by Mycobacterium avium complex (MAC) is one of the most common serious opportunistic infections in patients with AIDS. MAC is not killed by any standard antituberculosis drug except ethambutol at high level concentrations in plasma. For in vivo killing of MAC, the drug must penetrate macrophages as well as the MAC cell wall. In practice, there is a need for therapeutic strategies to be able to inhibit HIV and Mycobacterium replication that permit reducing toxicity and prolonging administration. Carrier erythrocytes containing prodrugs for slow delivery of AZT and ethambutol have been successfully tested in in vitro studies [110].

Efficient protection of the macrophages was also obtained with AZT prodrugs, such as AZTp2AZT, a new homodimer of AZT and acyclovir [108], and AZTp2ACV, a new heterodinucleotide of AZT and acyclovir, using carrier erythrocytes in murine AIDS model. AZTpPMPA, a heterodinucleotide of AZT and tenofovir (PMPA), is another prodrug of AZT which is slowly converted to PMPA, AZT monophosphate and AZT (half-life of 36 h at 37°C) by intra-erythrocyte enzymes and, therefore, is able to provide stochiometric amounts of both nucleoside analogues to macrophage cells and to overcome the low phosphorylating activity of monocyte-derived macrophages for AZT and the modest permeability of PMPA in vitro [246].

4.2.2.4.3. 2’,3’-Dideoxycytidine 5’-triphosphate
2’,3’-Dideoxycytidine 5’-triphosphate (ddCTP) is a nucleoside analogue that targets the reverse transcriptase of the HIV and is used in clinical management of AIDS. ddCTP, encapsulated in erythrocytes, may be used for selective targeting the drug to virus reservoirs as macrophages [247]. Although this drug in solution has the same antiviral activity as AZT, when administered encapsulated within erythrocytes it was several times more efficient in inhibition of human, feline, and murine immunodeficiency viruses [111,248].

4.2.2.4.4. 2’,3’-Dideoxycytidine prodrug
2’,3’-Dideoxycytidine (ddCyd) is one of the most potent antiviral nucleosides aimed for killing the HIV and is currently used in treatment of severe HIV infections. However, due to its rapid clearance, the drug must be administered frequently, thereby reaching toxic concentrations. Its prodrug, 5’-phosphate (ddCMP), encapsulated in erythrocytes, can be dephosphorylated by the intra-erythrocytic pyrimidine nucleotidase and, then, released as the active drug and, therefore, the carrier erythrocytes might be used as bioreactors for ddCyd delivery in treatment of HIV infection [249].

4.2.2.4.5. 9-(2-phosphonylmethoxyethyl) adenine
The main characteristic of 9-(2-phosphonylmethoxyethyl) adenine (PMEA) is its activity upon retroviruses, including HIV and the herpes virus (including HSV-1). This dual activity is of great importance in treatment of infections by retrovirus, as they are frequently complicated by opportunistic infections such as the herpes virus, which is the most common infection in these types of patients. Exposure of macrophages to PMEA-loaded erythrocytes inhibits the replication of both HIV and HSV-1 [104].

4.2.2.4.6. Adefovir dipivoxil (adefovir prodrug)
Adefovir dipivoxil (bis-POM PMEA), an oral prodrug of adefovir (PMEA), is formed by two molecules of PMEA joined together by a pyrophosphate bridge. This new prodrug was encapsulated in autologous erythrocytes modified to enhance their recognition and phagocytosis by human macrophages in vitro with favorable results [103].

4.2.2.4.7. 2-fluoro-ara-AMP (fludarabine)
2-fluoro-ara-AMP (fludarabine) is a fluorinated analogue of adenine that is useful in treatment of chronic lymphocytic leukaemia. The encapsulation of fludarabine in human erythrocytes in vitro permits a slow release that is prolonged for several days, whereby it is believed that it may be useful as a delivery system in treatment of malignant lymphomas using fludarabine [250].

4.2.2.4.8. 5-Fluoro-2’-deoxyuridine 5’-monophosphate (FdUMP)
Finally, 5-Fluoro-2’-deoxyuridine 5’-monophosphate (FdUMP) encapsulated in human erythrocytes may be used as bioreactors designed for time-programmed and liver-targeted delivery of the antineoplastic drug, 5-fluoro-2’-deoxyuridine (FdUrd) [251].

4.2.2.4.9. Heterodinucleotide of lamivudine and tenofovir
A novel heterodinucleotide including lamivudine and tenofovir which were revealed to have synergic effect were investigated through loading in the RBC. In this way, the modified heteronucleotide-loaded strategy was shown to be very efficient in protecting macrophages from de novo HIV infection [252,253].

4.2.2.5. Oligonucleotides, polynucleotides and their analogues
After initial experiments to evaluate the possibility of loading the genetic materials, such as DNA fragments (10), tRNA, and mRNA [254] in previous decades, recently, as a result of
considerable progress made in genomics, erythrocyte carriers are among several particulate drug delivery systems studied for the delivery of modern versions of nucleic acid-based biopharmaceuticals (e.g., antisense oligonucleotides, AODs) [255,256] and as non-viral gene delivery systems [257,258]. In recent years, peptide nucleic acid (PNA) antisense and anti-sense technology have featured in major medical applications [259-262]. Although PNAs have a considerable potential as antisense agents, their application requires efficient cellular uptake.

An established correlation is present between cytokine-induced nitric oxide synthase (iNOS) and demyelination in multiple sclerosis. Therefore, the Inhibition of iNOS using antisense oligonucleotides may give a promising strategy in multiple sclerosis treatment. It has been observed in studies in vitro, using murine macrophages, that the cellular uptake of antisense oligonucleotides may be favoured by using carrier erythrocytes. The in vitro opsonisation induced by ZnCl(2) and bis(sulphosuccinimidyl)suberate (BS(3)) have enhanced the phagocytosis of loaded erythrocytes and the delivery of PNA into macrophages. The efficacy of this carrier system in vitro has been demonstrated by decreased NO production and nitric oxide synthase (iNOS) protein expression in the macrophage [256]. Also, PNA-loaded erythrocytes can be used in order to block HIV release by targeting PNA into macrophage compartment. The main advantage of using erythrocytes is achieving efficient level of antiviral activity with lower concentration of PNA compare to use of free PNA demonstrating the potentiation effect of RBC as delivery system for PNA [263].

Byun et al. have reported the use of erythrocyte ghosts as a biocompatible non-viral delivery system for extended circulation and prolonged expression of murine interleukin-2-xpressing plasmid DNA in the blood, using electroporation as the encapsulation method. 21 min after IV administration to mice, the level of plasmid DNA in the blood was 92000-fold higher following erythrocyte ghost-mediated delivery as compared to the injection of naked form. In the same study, erythrocyte ghost-mediated gene delivery revealed higher and more prolonged mRNA expression levels of plasmid DNA in the blood until 9 days after a single IV injection. Moreover, plasmid DNA-loaded erythrocyte ghost showed specific gene expression in the blood cells. At three days post-dose, substantial expression levels of plasmid DNA delivered within erythrocyte ghost were observed only in the blood and not in the other organs. The results indicate the potential of erythrocyte ghost as a safe, prolonged and blood-targeted delivery system of therapeutic genes [258].

In another study reported recently by this group [264], opsonized erythrocyte ghosts have been successfully used for liver-targeted delivery of antisense oligonucleotides (AODs). For opsonization, the AODs-entrapped erythrocyte ghosts were incubated with rabbit anti-mouse RBC antibody for 1 hour. Opsonized erythrocyte ghosts encapsulated with AODs exhibited a mean residence time significantly shorter than control. The biodistribution of this carrier system depend on opsonization, with opsonized carriers producing 4.5-fold higher levels of AODs in the liver compared with unopsonized carriers. Collectively, the authors concluded that opsonized erythrocyte ghosts can be used for liver-targeted delivery of AODs.

5. OTHER APPLICATIONS

5.1. Improvement of oxygen delivery to tissues
Inositol hexaphosphate, with higher binding affinity to hemoglobin (Hb) compared with 2,3-di-phosphoglycerate, is an allosteric effector of Hb that has a limited capacity for penetration in the erythrocytes. The incorporation of inositol hexaphosphate in erythrocytes produces a modification in Hb-oxygen affinity, increased O2 release and reduces cardiac output. After encapsulation in erythrocytes, it is irreversibly joined to the intracellular hemoglobin and as a result of its action it increases the transport of O2 and CO2 between lung and tissues. This effect is particularly important for different therapeutic applications, such as ischemic pathologies in myocardium, brain and other tissues. Some authors have studied the consequences of a substantial and long-term increase of the in vivo partial pressure of oxygen in piglets after exchange transfusion with inositol hexaphosphate enriched erythrocytes. The physiological modifications, such as reduced cardiac output in the absence of any other effects detectable, suggest the possible use of these inositol hexaphos-phate-loaded erythrocytes to restore normal oxygenation of impaired blood flow [265-267]. More details about the suggested applications of this system could be find in reference 20.

5.2. Preparation of fused cells
Another fascinating application of carrier erythrocytes, though less developed, is their use in the injection of different compounds into eukaryotic cells and cytoplasmic compartment of tissue culture cells. In this procedure, the erythrocyte membrane merges with that of the host eukaryotic cell by means of the fusogenic agent and the content of the erythrocyte, including the previously encapsulated substance, is transferred to the host cell [269]. This method has been tested in vitro for the microinjection of arginase, fragments of DNA, nucleic acids, ferritine, albumin, and thymidine kinase to different types of eukaryotic cells [270,271]. The main advantage of this method compared to endocytosis is avoiding the degrading effects of the lysosomal enzymes on the injected compounds [271].

5.3. Metabolism studies by red blood cells
There are some reports on using erythrocytes as cellular models to the metabolism of encapsulated compounds in the erythrocyte or to observe the effect of assumed substances on a normal cell. Within this context, various studies have been designed in order to study the metabolic influence of glucose 1.6-biphosphate in red blood cells using human erythrocytes loaded with this molecule [272]. Also some enzymes such as hexokinase and glucokinase have been encapsulated in erythrocytes to study their role in the metabolism of the erythrocytes when there are in enzymatic deficiencies [30,32,273-276]. As another example, glucose oxidase of Aspergillus niger has been encapsulated in human erythrocytes as a model system for cytotoxicity studies [30].

6. THE NEXT GENERATION OF ERYTHROCYTE-BASED CARRIERS

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Although erythrocytes represent a unique ad promising platform for drug delivery purposes, however, carrier erythrocytes are still somewhat underdeveloped. In the other word, after initial interests and studies three decades ago, carrier erythrocytes were overshadowed by artificial carriers mainly due to some intrinsic limitations of biological carriers like erythrocytes (discussed in Table I). Therefore, the new generations of erythrocyte-based carriers, which will be discussed below, are, in fact, the modern answers for these drawbacks.

### 6.1. Nanoerythrosomes

To address the limitations of carrier erythrocytes as well as other drug carriers (like liposomes and monoclonal antibodies), Gicquaud and coworkers, have developed a new erythrocyte-based drug carrier called nanoerythrosome (nEryt) with promising results [277]. which are vesicles prepared by the extrusion of red blood cell ghosts with the average diameter of 100 nm. Therefore, they can freely extravasate from endothelium and can reach extravascular targets inaccessible to intact erythrocytes. Daunorubicin was covalently linked to nEryts using glutaraldehyde as homobifunctional linking arm and the cytotoxicity [277], antitumor index [278,279], mechanism of action [280] of this system was studied in vitro and in vivo. nEryt-conjugated daunorubicin showed higher antiproliferative index than the free drug [279] and after intravenous administration, they were rapidly (<30 Min) removed from blood circulation by RES organs [280]. These nanocarriers offer a high degree of adaptability for the encapsulation of various biological or non-biological compounds and for the binding of targeting agents. In particular, PEG can be conjugated by a covalent link to the basic amino acid residues constitutive of different proteins. The binding of PEG to the nEryt membrane could be interesting for the therapeutic use of this delivery system since it could overcome heterologous immunogenicity and reduce rapid clearance from circulation [281].

### 6.2. Erythrocyte-adsorbed nanoparticles

Polymeric nanoparticles have been extensively studied for use as intravascular drug delivery vehicles; however, their applications are restricted because of rapid clearance from circulation by RES. Previous endeavors to improve vascular circulation have focused on surface modification using polymers such as poloxamines, poloxamers, and polyethylene glycol, to prevent opsonization. However, Mitragotri and coworkers [282] have reported a novel method of prolonging intravascular particle circulation by anchoring the nanoparticles to the surface of erythrocytes. Their hypothesis, motivated by the strategy adopted by hemobartonella that adhere to erythrocytes and remain in circulation for several weeks, has been proved with RES escape and prolonged circulation of nanoparticles as large as 450 nm in vivo. The particles have remained in circulation as long as they remain attached to erythrocytes. Although particles were eventually detach from carriers and eliminated from circulation, erythrocytes were not cleared [283]. Erythrocyte-anchored nanoparticles offer a novel approach for intravascular drug delivery and blood pool imaging.

### 6.3. Versatilized biotinylated erythrocytes

As mentioned in section 3.2.2, membrane association of pharmaceuticals, especially biopharmaceuticals, by means of avidin-biotin bridges is the most widely used strategy for non-encapsulation loading of erythrocyte carriers with bioactive agents [25,91]. Controlled and non-damaging biotinylation of intact mammalian erythrocytes could lead to a generalized and versatilized circulating carrier. In particular, controlled biotinylation of erythrocyte lysine residues using NHS esters of biotin become arguably one of the most popular means for conjugation cargos to erythrocyte surface via avidin-biotin cross-linker for a wide variety of applications in vitro and in vivo. In vivo studies demonstrated that an erythrocyte carrying up to 105 molecules of protein cargo conjugated via streptavidin circulates in animals similarly to naive erythrocyte, without enhanced clearance, lysis or organ uptake [24,91]. Furthermore, methods for direct biotinylation of erythrocyte in the bloodstream using intravascular injection of biotin esters have been explored [284].

### 6.4. Antibody-guided loading of circulating erythrocytes

More recently and to address the safety and translational concerns relative to the ex vivo loading of drugs in/on erythrocytes as carriers, some groups have proposed drug delivery systems targeted to anchor molecules on erythrocyte surface using erythrocyte-specific surface ligands, and therefore, allowing safe and technically simple loading of circulating erythrocytes to expand general applicability of carrier erythrocytes in medicine. The first approach used Complement Receptor Type 1 (CR1) anchoring strategy, since therapeutics and non-therapeutic agents conjugated with monoclonal antibodies to CR1 bind to circulating erythrocyte after IV injection an animal models, circulate with erythrocyte and bind ligands in vivo without erythrocyte damage [285,286]. Animal studies showed that tPA conjugated to a CR1 monoclonal antibody binds without harm to circulating erythrocytes in mice and affords safe and effective prophylactic thrombolysis [287] comparable to that provided by infusion of RBC/tPA system [229]. To alleviate challenges associated with translation and application of chemically produced antibody/tPA conjugates and achieve predictable loading of erythrocyte over a wide range of drug concentrations, Murzykantov and coworkers have developed a novel recombinant tPA mutant fused to an antigen-binding single chain variable fragment (scFv) of TER-119 monoclonal antibody to glycoporin A (anti-GPA scFv) to load up to 20,000 copies of anti-GOA scFv/tPA fusion per erythrocyte after a single IV injection of the fusion without detectable changes in behavior of circulating erythrocyte. Injection of scFv/tPA in mice provided swift dissolution of subsequently formed intravascular clots, which were impervious to injections of equimolar doses of soluble tPA [288]. In another study by this group [289], scFv was fused with a variant human single-chain low molecular weight urokinase construct that can be activated selectively by thrombin (scFv/uPA-T). scFv/uPA-T bound specifically to mouse erythrocytes without altering their biocompatibility and retained its zymogenic properties until converted by thrombin into an active two-chain molecule. As a result, erythrocyte-bound scFv/uPA-T caused thrombin-induced fibrinolysis. A single IV
injection of the fusion without detectable changes in behavior of circulating erythrocyte. Injection of scFv/tPA in mice provided swift dissolution of subsequently formed intravascular clots, which were impervious to injections of equimolar doses of soluble tPA [288]. In another study by this group [289], scFv was fused with a variant human single-chain low molecular weight urokinase construct that can be activated selectively by thrombin (scFv/uPA-T). scFv/uPA-T bound specifically to mouse erythrocytes without altering their biocompatibility and retained its zymogenic properties until converted by thrombin into an active two-chain molecule. As a result, erythrocyte-bound scFv/uPA-T caused thrombin-induced fibrinolysis. A single IV injection of scFv/uPA-T provided effective prophylaxis against arterial and venous thrombosis for up to 24 hours. The authors concluded that prophylactic delivery of erythrocyte-targeted plasminogen activator produgs activated selectively at the site of clot formation represents a new approach to prevent thrombosis in clinical setting where the risk of clotting is high.

6.5. Artificial erythrocytes
As discussed as a drawback of erythrocyte carriers, being from biological origin, entrapped erythrocytes may present variability and lesser standardization in their pilot and large scale preparation, compared to other synthetic carrier systems. Also, there are many safety and technical concerns related to the storage of the loaded erythrocytes due to their liability to biological contamination [20,21]. On the other hand, biomaterials form the basis of current and future biomedical technologies which are routinely used to design novel therapeutic carriers, such as nanoparticles, for applications in drug delivery. Current strategies for synthesizing drug delivery carriers are based either on discovery of materials or development of fabrication methods. While synthetic carriers have brought upon numerous advances in drug delivery, they fail to match the sophistication exhibited by innate biological entities. In particular, erythrocytes, the most ubiquitous cell type in the human blood, constitute highly specialized entities with unique shape, size, mechanical flexibility, and material composition, all of which are optimized for extraordinary biological performance. Inspired by this natural example, Mitragotri group has synthesized particles that mimic the key structural and functional features of RBCs. Similar to their natural counterparts, RBC-mimicking particles described recently possess the ability of carrying oxygen and flow through capillaries smaller than their own diameter. Further, they can encapsulate drugs and imaging agents. These particles provide a new paradigm for the design of drug delivery systems and imaging carriers, because they combine the functionality of natural erythrocytes with the broad applicability and versatility of synthetic drug delivery particles [23].

7. CONCLUDING REMARKS AND FUTURE HORIZONES
Carrier erythrocytes are naturally-designed, promising and multi-purpose drug delivery systems. After more than three decades of efforts and studies for their development they are still underdeveloped delivery platform. However, considering recent advances in this field, several completed and running clinical trials, and novel approaches presented as “the next generation of erythrocyte-based carriers”, it could be concluded that we would see a developmental revolution and paradigm shift in erythrocyte-based drug delivery platform in the near future.

References

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117. Vyas SP, Jain SK. Preparation and in vitro characterization of a magnetically responsive ibuprofen-loaded...


