A COMBINATION OF CARRIER ERYTHROCYTES AND ARTIFICIAL NANOPARTICLES AS A PROMISING APPROACH FOR DRUG DELIVERY

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The approaches and engineered techniques for safely carrying a pharmaceutical compound in the body to achieve its desired therapeutic effects are known as drug delivery systems. Increased awareness that drug release patterns can affect therapeutic responses, the necessity of safe and efficient drug administration, and the requirement of novel strategies to deliver complex drugs fueled the drug delivery research. Some active pharmaceutical compounds also show low bioavailability, low water solubility, and membrane permeability. Scientists have understood that drug safety and efficacy can be improved, and new therapies are possible when a drug is encapsulated within or attached to a carrier. A drug carrier is any substance that improves drug administration's selectivity, drug release pattern, and effectiveness. Capsules, liposomes, micelles, and nanoparticles are examples of drug carriers. It became clear that the drug carrier systems are essential as the drug itself. Nanotechnology's application in drug delivery is reported to improve the therapeutic outcomes of various diseases. Even for highly lethal cancers where a cure in the past was beyond our reach. Nevertheless, challenges related to biocompatibility, cytotoxicity, and rapid clearance limited the use of nanomedicine. Parameters such as shape, particle size, surface properties, and charge are optimized to overcome those challenges. As a result of extensive research, erythrocyte membrane camouflaged nanoparticles loaded with drugs have become an attractive candidate for drug delivery. Nanocarriers such as mesoporous silica nanoparticles, magnetic nanoparticles, and solid lipid nanoparticles are now successfully employed in the combined strategy. The combined strategy has offered an opportunity to unite natural cell membrane properties with artificial nanoparticles. The coated nanoparticles are proved to ensure biocompatibility, extended circulation, execution of targeted functions, and safety. Up to now, few reviews have discussed the complete picture of this combined drug delivery strategy. This article reviews the background, development, and importance of the combined strategy, and provides a foundation to stimulate the interests and understandings in this novel strategy.

Keywords: Biocompatibility, Biodistribution, Co-delivery, Membrane coated nanocarriers, Targeted drug delivery.

1. Introduction

Drug delivery is a research field investigating the methods or processes of administrating a pharmaceutical compound to result in a therapeutic effect in humans or animals [1]. Drug delivery systems evolved to address the challenges associated with conventional drug administration. A known fact is that the traditional drugs are administrated as capsules, oils, or creams where the therapeutic compounds are frequently released early upon contact with water (e.g., tablets), captured by the immune system, or will not reach the desired target. Many brain diseases are poorly treated, and new drug development rates are low due to drugs' inability to cross the blood-brain barrier proves conventional drugs' inability to reach a specific target [2]. Also, the need for efficient drug delivery in enhancing the efficacy of anti-retroviral drugs is felt. The invention of retroviral drugs only did not significantly help to treat patients. The requirement of novel drug delivery systems, especially capable of carrying the antiviral medication to the targeted site, is essential (e.g., antiviral drugs capable of penetrating the blood-brain barrier to treat HIV-1 encephalitis) [3]. However, increasing the drug dosage is not the right solution and can result in unexpected side effects in normal tissues. These challenges fueled the research focused on efficient drug delivery systems, including nanocomposite systems [4], liposomal drug delivery [5], therapeutic molecules linked to polyethylene glycol (PEG) [6], and circulatory cells modified as carriers (e.g., erythrocytes) [7]. It became clear that many pharmacological properties of conventional "free" dugs can be enhanced by employing a drug delivery system. Drug delivery systems usually alter the pharmacokinetics and biodistribution or bioavailability of the associated pharmaceutics agent or function as drug reservoirs, or both [8].

The vehicles used to deliver the therapeutic compound to the target site are drug carriers. Recently, nanocarriers have become a promising candidate for effective drug delivery, but they are debatable. In oncology, nanotechnology has contributed to achieving deep tumor penetration, effective protection of therapeutic compounds against many obstacles, better tumor selectivity [9], and reduced systemic accumulation [10]. Co-delivery of nanoparticles to deliver suitable drug pairs became extremely attractive in the medical field [11]. The intervention of nanotechnology has altered the opinion that the carriers are just an excipient in drug delivery. Modified micellar nano-complex carriers are equipped with therapeutic effects (anticancer effects) [9]. These benefits from nano-based concepts can reduce the dose required to achieve a specific therapeutic response [12]. Nanomedicine's increased complexity has brought about significant outcomes and issues such as bio incompatibility

and toxicity [13]. The strong complement activation was seen with some nanomedicine administration as a significant challenge that needs to be addressed [14]. Rapid clearance of nanoparticles by macrophages [15] limits the biodistribution and availability of nanocarriers loaded with drugs. Due to this, expected therapeutic responses are not observed. It became clear that the real challenge is not just discovering new drug delivery systems, but the ones with high safety, efficacy, and specificity. At present, numerous trials and errors have resulted in advances in drug delivery systems.

Inspired by several strong points associated with circulatory cells such as erythrocytes [16], researchers started to incorporate these natural drug delivery carriers to avoid accelerated blood clearance and IgM (immunoglobulin M) response [17]. Studies present the potential of drug delivery carriers that mimic erythrocytes' functional and structural properties in addressing major challenges faced by current drug delivery systems. Biodistribution and biocompatibility of such modified systems were promising [18]. A combined strategy comprising synthetic nanoparticles and natural erythrocytes is now realistic and reported to show promising results. Advanced techniques that bypass labour-intensive purification steps and conjugations help produce erythrocyte membrane camouflaged nanoparticles showing long circulation times [19]. Novel methods for preparing erythrocyte ghosts, drug loading, and resealing erythrocytes help assure the success of this combined approach in the medical field [20]. Been less influenced by nanocarriers' morphology and size, the combined strategy allows various nanocarriers in effective drug delivery. Some of them are gold nanocages [21], Fe_3O_4 nanoparticles [22], and mesoporous silica nanoparticles [23]. Interestingly, with the help of erythrocyte vesicles, this approach is applicable in several treatment procedures targeting the reticuloendothelial system [24] spleen and liver [25], and toxin vaccination [26]. This proves that the modified approach can expand efficient drug delivery systems in treating severe disease conditions without being restricted to cancer treatments. Assuring the safety of the drug carriers is equally essential. In vitro cytotoxicity assays [27] and verification of surface proteins [28] have provided evidence to prove the combined system's safety. However, this approach's future challenges have to be identified and addressed to transform the preclinical efforts into functional systems. Different strategies have to be tested instead of the same approach tested for more extended periods with poor outcomes. This review will show the requirement of drug delivery systems' involvement modified using nanotechnology and cell-based delivery systems to address the prevailing issues in drug delivery, and provides the complete picture of this combined drug delivery strategy.

2. Drug development and associated challenges

The origin of drug therapy was linked with the dye chemistry's evolution (1872-1874), mainly due to the understanding of the selective affinity of dyes for biological tissues led by Paul Ehrlich. This led to the argument that certain chemoreceptors found on cancer cells and microorganisms are different from analogous structures found in hosts. These differences can be employed in therapeutics [29]. Usually, identifying a biological target such as an enzyme, receptor, or gene found to be dysfunctional in patients with a specific disease leads to developing a new drug. A biological target will help develop drugs, that influence the target in a therapeutically useful way [30]. So, in addition to chemistry, pharmacology, and later understandings of molecular biology have also helped to shape the process of drug discovery. Molecular biology extended the understanding of disease processes at the molecular level, which helped determine the molecular targets for drug intervention [29].

The drug development process is costly, mainly because of the intense research and development and clinical trials. Costs are trended upward for decades. A study on self-originated new therapeutic compounds that have first entered clinical testing around 1993-2004 through June 2009 uncovers that approximately one in six medical drugs that reach the clinical testing pipeline will finally achieve marketing approval in the United States [31]. Many drugs do not step through this entire process due to the problems that arise with safety, lack of sufficient evidence to prove biologic activity, and kinetics potency [30]. Studies report low success rates of drugs to treat brain disorders due to the lack of efficient drug delivery systems to deliver pharmaceutical compounds to the brain [2]. These facts have led to specific trends in low success rates in drug approval, where drugs can leak out of the pharmaceutical pipeline at each step of the process of drug development and never make it to market (Table 1).

Table 1. Estimated phase transition and clinical approval success rates by therapeutic class for self-originated drugs (originated from the pharmaceutical company itself) first tested in humans from 1993 to 2004 [31].

Therapeutic class	Phase I- II (%)	Phase II- III (%)	Phase III-Regulatory review (%)	Regulatory review-approval (%)	Clinical approval success rate (%)
Cardiovascular	62.9	32.4	64.3	66.7	8.7
Musculoskeletal	72.4	35.2	80.0	100.0	20.4
CNS	59.6	33.0	45.4	90.0	8.2
Respiratory	72.5	20.0	85.7	80.0	9.9
Systemic anti-infective	58.2	52.2	78.6	100.0	23.9

3. Drug delivery and importance

Drug delivery can be explained as a research field that works to develop a method to administer drugs to the desired site of the body to accomplish a therapeutic impact by overcoming the challenges associated with conventional drug administration. Conventional drug administration forms depend on tablets, eye drops, ointments, creams, and intravenous solutions. However, the need for the safe and efficient administration of drugs, complications associated with newer and complex drugs, and the dependence of therapeutic responses on drug release patterns (continuous or pulsatile release) catalyzed the curiosity and research on new drug delivery systems [32]. It is crucial to find methods to administrate a drug at a safe dosage that maintains the accurate concentration of a drug at the site of action. The need for efficient drug delivery systems is felt in several disease treatment procedures. The cancer field studies have suffered a lot due to the lack of good drug delivery systems. A colorectal cancer statistics-based study shows a declining trend in colorectal cancer mortality in both men and women. They suggest that the main reason is the advancements in early diagnosis and treatments. The need for advanced treatment methods for tumor subtypes that show an inadequate response to current therapies is uncovered [33]. Drug delivery to the eye can also be presented as another example. Here, eye drops are the most popular dosage form of the ocular route. But the innate protective mechanisms and continuous bathing of tears barrier the efficient drug delivery. The incorporation of nanotechnology knowledge has allowed efficient intraocular absorption of drugs with enhanced delivery to the back of the eye [34]. To further support the title “the importance of drug delivery,” the development of active targeted drug delivery to effectively arrest abdominal aortic aneurysm using ligand-receptor binding (surface adhesion of drug incorporated microbubbles to inner walls of abdominal aortic aneurysm, which is an irreversible bulge observed in the arteries) is already studied together with factors that affect better performance [35].

4. Evolution of drug delivery systems

The development of drug delivery systems can be addressed by dividing it into three generations as discussed below.

4.1 First-generation drug delivery systems

The discovery of the first microencapsulated drug particles in 1952 is considered the first-ever drug delivery technology. This is known as Spansule® technology and developed to

achieve a 12-hour drug release. By 1960, polymer utilization was ubiquitous in drug delivery [36, 37]. Later in the 1970s, scientists understood the importance of developing a zero-order (drug released at a constant rate) pharmacokinetic profile. Several drugs (e.g., cardiovascular drug nifedipine, also known as Procardia), with better efficiency, were developed with these understandings [36]. In 1991, the effects from immediate-release, sustained-release, and intravenous administration of the drug nifedipine were studied, and results presented [38]. The progress made after 1950 was mainly regarding the quality of the technology and clinical formulations, which comprise the first-generation drug delivery systems [37].

4.2 Second-generation drug delivery systems

The second-generation drug delivery systems (1980-2010) were self-regulated with zero-order release rates and nanotechnology-based. Still, success in discovering clinical formulation was low due to the inability to overcome biological barriers and to remain stable. The limited success with second-generation drug delivery systems showed the requirement of both analytical and attentive analysis in current technologies (third-generation drug delivery) for successful clinical applications [36, 37].

4.3 Third-generation drug delivery systems

The current generation of drug delivery systems, also known as third-generation drug delivery, has continued since 2010. This is primarily based on conjugated polymer-drug development, and nanotechnology employed targeted drug delivery. Nanocarriers such as micelles, dendrimers, liposomes, and hydrogels are widely applied to achieve efficient drug delivery. The third-generation drug delivery system has defeated most of the complications in the first- and second-generation formulations [36].

The third-generation modulated drug delivery systems can be discussed under three broad topics, and some of these delivery technologies are developing from the second generation.

4.3.1 Self-regulated drug delivery

These systems are based on smart polymers with various active groups capable of recognizing and responding appropriately to the changes in normal physiological functions. Similarly, stimuli-responsive polymers are also studied in drug delivery. pH-responsive polymers that release the encapsulated drugs in response to low pH values are promising drug carriers [39]. pH-sensitive hydrogels with immobilized glucose oxidase and catalase

for self-regulation of insulin release for diabetes treatments are good examples of this type of delivery system. In this case, glucose oxidase will catalyze glucose conversion to gluconic acid, thereby lowering the pH and resulting pH-sensitive hydrogels to collapse [40]. Similarly, multi-responsive micelles with low cytotoxicity and enhanced stability, capable of releasing insulin appropriately under different glucose concentrations, are also studied. The results have uncovered the possibility of employing these kinds of micelles in repeated and efficient on-off drug release [41].

4.3.2 Delivery of poorly water-soluble drugs

An analysis carried on top 200 oral drugs showed that; a considerable fraction of drugs (approximately 30-40%) were with low solubility. Many new drug candidates show poor water solubility. This can result in a large portion of the drug not been translated into clinically useful formulations [42]. Poorly water-soluble drugs require high doses to reach plasma concentrations with therapeutic effects. To overcome these challenges, micellar solubilization [43] is successfully tested to enhance drugs' solubility.

4.3.3 Targeted drug delivery

Currently, nano-assembled drug delivery systems capable of targeted drug delivery are tested for effective anticancer therapy. Nano-assembly loaded with anticancer drugs such as poly-cyclodextrin and poly-paclitaxel, targeting specific proteins, has shed light on cancer treatments [44]. Advanced techniques such as magnetic navigation help efficient targeting and avoid off-target effects [10]. Liposome in targeted drug delivery is also studied [45]. The need for better-targeted drug delivery and advanced techniques (e.g., ultrasound controllable drug carriers) is highly felt in brain disease treatments where off-targeting can be severe [46]. Similarly, some other strategies, including site-specific drug-DNA conjugates (synthetic drug-DNA adducts) showing anticancer effects with high selectivity, resistance to nuclease activity, and increased drug payload capacity, are studied [47]. These are few examples out of many to prove the importance and need for targeted drug delivery. From these study objectives and results, it is clear that targeted delivery is crucial for increasing the therapeutic outcomes of compounds by improving the delivery to the target site with less accumulation in normal healthy tissues (Fig 1).

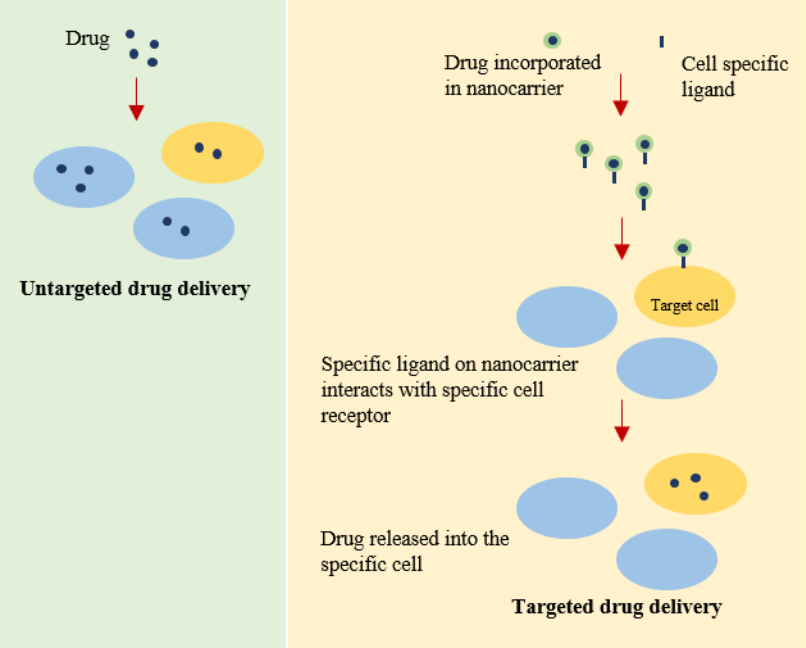

Fig 1. Targeted drug delivery. Nanocarriers carry the therapeutic compound into the targeted cells using ligand-mediated binding, with high selectivity. This avoids the accumulation of the drug in nonspecific cells/targets.

5. Nanotechnology and its impact on drug delivery

The National Nanotechnology Initiative (NNI) explains nanotechnology as the science, engineering, and technology of matter at a nanoscale (1-100 nm). Nanotechnology involves studying and applying nano entities in other science fields such as biology, engineering, and physics [48]. The initial idea and concept of nanotechnology came into existence with a talk titled "There's Plenty of Room at the Bottom" by Richard Feynmann, a physicist [49]. Currently, nanotechnology has spread its roots in several fields. One such offshoot of nanotechnology is nanomedicine. Scientists who have been dedicated to elucidating the basis and possible strong points of nanomedicine show that modifications can enhance site selectivity, maintain dose, accumulation at target sites, and reduced side effects. Molecular docking simulation techniques involving computer design to synthesize nanocarriers help to customize nanocarriers [50]. Also, biological understandings such as the enhanced permeability and retention (EPR) effect studied by Yasuhiro Matsumura and Hiroshi Maeda in 1986 [51] have now become handy to passively and continuously accumulate nanoparticles in tumor sites [52]. Currently, targeted drug delivery using nano systems are attractive and extensively studied [53]. The past studies are also crucial in the development of novel nanomedicines. Studies related to lipid vesicles' structural identifications, now known as liposomes (type of nanocarrier), were published in 1964 [54]. Ultimately modified

biomimetic nano drug delivery systems now show long-term circulation in the body, ability to cross biological barriers, targeting specific sites, and reduced tissue toxicity [55].

5.1 Active targeting with nanotechnology

Targeted delivery of therapeutic compounds is possible with nano drug delivery systems. By 2021, appropriately modified solid lipid nanoparticles capable of crossing blood-brain barrier and targeting neurons to treat Alzheimer's disease are reported [53]. Targeting specific cells or tissues using ligand-conjugated nanocarriers increases the therapeutic efficacy of relatively small molecules. It increases their cellular uptake, unlike the traditional nonspecific drug delivery that damages normal healthy cells. The EPR effect is employed in nanotechnology to make drug delivery advancements in cancer treatments [56]. Active targeting has given the hope to modify treatment methods for brain diseases by overcoming the challenges associated with the blood-brain barrier, a selective barrier formed by microvascular cells connected with tight junctions [57]. In 2016, T. Lin and others developed an efficient method for synthesizing albumin nanoparticles with different co-encapsulated drugs capable of penetrating the blood-brain barrier [58]. Ligand-conjugated carriers show higher frequencies of encountering the targeted cells through a receptor-mediated endocytosis process. Nano-assembly of polymeric cyclodextrin and paclitaxel with enhanced stability, efficient uptake by specific cancer cells, and no harmful effects on normal cells is an excellent example. In this study, AP-1 peptide targeting an interleukin-4 receptor overexpressed on some cancer cell types is incorporated into nanocarriers [44]. Moreover, tumour diagnosis and tumour targeting properties of glycogen based-nanopolymers are reported. Additional modifications have reduced the uptake of these nanopolymers by internal organs, lowering side effects [52]. Targeted nanocarriers can also act as gene vectors in cervix cancer therapies. Folate receptor-mediated targeting is used to deliver pigment epithelium-derived factor (*PEDF*) gene coding PEDF protein that shows anti-tumor properties [59].

5.1.1 How nanocarriers carry drugs into the targeted sites?

Delivery of drug-loaded nanocarriers to the targeted site or the cell can be achieved through ligand incorporation [44]. Specific peptides can be used as cell-specific targeting agents [60]. A study has uncovered nanocarriers' capability, such as pH-sensitive liposomes, to carry therapeutic agents such as N-butyldeoxynojirimycin (a potent depigmenting agent used in hyperpigmentation disorders) across the plasma membrane of a cell. According to the results,

these liposomes are taken up by the cells through endocytosis. By optimizing the composition of these carriers, they could be destabilized in the acidic endosomes. This can finally result in the release of the drug-loaded inside the carriers into the cytoplasm and endoplasmic reticulum at less toxic concentrations [5]. Another study uncovers the ability of pH-sensitive micelle conjugated with TAT peptide to carry any hydrophobic drug near the nucleus [61]. This way, nanocarriers can carry drugs into specific cells or even to particular organelles inside the cells without damaging the normal cells.

5.2 Co-delivery of drugs using nanotechnology

Especially in cancer treatments, co-delivery can effectively inhibit tumor growth. Co-delivery of Palbociclib (a CDK4/6 inhibitor) and hydroxychloroquine (an autophagy inhibitor) has shown outstanding results in pancreatic cancer treatment [11]. Doxorubicin (Adriamycin) is widely used in cancer chemotherapy, including in B cell lymphoma. Nevertheless, it is a nonselective cytotoxic drug that limits its administration in combination with other anticancer drugs. Interestingly, polymer nanomaterial optimization to carry curcumin (showing a synergistic effect with several other anticancer drugs) and doxorubicin are possible. This combination shows high anti-lymphoma impact and low toxicity [62].

5.3 What makes nanotechnology a false promise in drug delivery?

Potential side effects of nanomedicine can be predicted using already available knowledge on free drugs, the behavior of different nanomedicine approaches, and their interactions with biological components and systems. Increased complexity of nanomedicine can lead to bio incompatibility, toxicity, and other safety issues [13, 14, 45].

5.3.1 Toxicity and pseudoallergy

Arguments arise regarding the suitability of in vitro studies on nanoparticle toxicity, as it might not be an adequate substitute for whole animal studies. However, in vitro studies can still uncover the basis for further assessing nanoparticles' potential toxicity risks. One such understanding from in vitro studies is that oxidative stress is likely to contribute to nanomaterials' cytotoxicity [13]. Another research has revealed that smaller nanoparticles lead to higher toxicity due to the larger surface area per given mass. For some nanoparticles, the treatment duration can also be a significant parameter associated with cytotoxicity [63]. Complement activation-related pseudoallergy limits the clinical use of many nanomedicines.

Studies show that the complex complement activation mediated by nanoparticles depends on the particles' surface properties in addition to their charge [14].

5.3.2 Other biological challenges

Understanding the association between biology and technology is crucial to point out the biological challenges in nanomedicines' clinical translation. Most of the papers regarding nanotechnology in the medical field are based on tumors, and the clinical translation of most of the published studies is low. These can pose the question, "Is nanotechnology not good to solve other medical problems." The ability of nanoparticles to cross the blood-brain barrier can also be discussed under this. Some studies report the blood-brain barrier dysregulation and impact on neural activity due to the exposure to environmental nanoparticles (e.g., TiO_2 nanoparticles) [64]. Moreover, nanocarriers are recognized as 'nonself' and are quickly removed by body's immune system [55]. Nanocarriers such as liposomes are involved in IgM absorption. This results in rapid nanocarriers' clearance, reducing the nano agent's efficiency and bioavailability [45]. Especially in the biological fluid, nanoparticles become coated with proteins and form a protein "corona" that will define nanoparticles' biological fate [65]. The presence of complement proteins and apolipoproteins with immunoglobulins in the protein corona is observed, and their involvement in triggering the clearance of nanoparticles is already understood [66].

5.3.2.1 How do nanoparticles get cleared in vivo?

Nanoparticle clearance mainly takes place via complement activation and lipid trafficking pathways [66]. Frequently IgM is secreted by B-cells bound onto nanoparticles [45]. IgM is secreted as the first line of defense, usually during an infection. With this, activation of classical complement pathway and complement component 3 (C3) deposition on nanoparticles' surface contributes to the uptake of particles by macrophages mediated specific ligand-receptor interactions [15]. C3b fragments act as opsonins to enhance the uptake and removal of foreign particles (identified as invaders) by phagocytes. C5a fragments also help in activating phagocytose. After phagocytosed, membrane-bound phagosomes are formed, followed by the fusion with lysosomes [67]. Phagolysosomes are with a pH low as 3, at which synthetic, biodegradable (a requirement in pharmaceutical applications) polymeric nanoparticles get degraded [68]. This has made researchers rethink the suitability of nanotechnology's application alone and the necessity of modifications to make it useful in the medical field.

6. Cell-based drug delivery platforms

Nanoparticles used to enhance the therapeutic efficacy can comprise several challenges: degradation of a therapeutic agent, phagocytic clearance, reduced circulation time, and sometimes poor targeting. Circulating cells or their membranes can aid in minimizing some of these challenges accompanied by nanomedicine. Cells such as platelets [69], erythrocytes [70], macrophages [53], leukocytes [71], and some other cell types are used successfully to modify nanocarriers (Table 2). Different proteins and carbohydrates on these cell membranes involve in a variety of body functions. Therefore, nanocarriers coated by such cell membranes inherit the features such as immune escape, and long circulation [55]. Carrier erythrocytes were studied since 1979 [72] as a drug carrier system (e.g., enzyme-loaded erythrocytes). As suggested, Enzyme-loaded erythrocytes are phagocytosed by macrophages, and this is employed to deliver enzymes into the bone marrow. Also, platelets can be employed as drug carriers, supported by the fact that they can act as carriers of active proteins during an injury. Platelets loaded with doxorubicin as a smart drug delivery system for lymphoma provide evidence [69].

6.1 Carrier erythrocytes as drug delivery systems

Erythrocytes mainly involve in O_2 and CO_2 transport between the lungs and tissues. Erythrocytes possess a life span of 120 days, and worn-out erythrocytes are phagocytosed and later digested by macrophages, usually in the liver and spleen [73]. In recent years, erythrocytes' application as a potential site-specific or slow drug release system has earned significant attention in the medical field. Erythrocytes act as biocompatible and biodegradable carriers for a range of bioactive agents with a series of proposed mechanisms for drug release, including phagocytosis and then release of the drug into circulation by passive diffusion or by the association of specialized agents on to the membrane [16]. The association between therapeutic compounds and erythrocyte carriers can be successfully achieved through two efficient approaches. Drug encapsulation in erythrocytes is the most prominent approach, and the other approach is the attachment of the nanoparticles onto the erythrocyte's membrane [74]. The availability of advanced and efficient techniques and enough understandings have helped to use erythrocytes as drug delivery carriers. One such success story reported in 2001, the effectiveness of an erythrocyte-mediated delivery strategy of dexamethasone to patients with chronic obstructive pulmonary disease. The lysed erythrocytes in a hypotonic solution and resealed them after incorporating dexamethasone 21-phosphate [70].

6.2 What makes erythrocytes a potential carrier?

Although nanoparticles are frequently tested as potential drug delivery systems, several challenges need to be overcome. Erythrocytes can be used as "supercarriers" of nanoparticles as they can significantly alter the biodistribution of nanoparticles [74]. The potential to address several challenges accompanied by current drug delivery carriers is observed in drug delivery carriers that mimic erythrocytes' properties. Carriers mimicking the shape, size, ability to deform, and elastic modulus of erythrocytes are reported to have enhanced drug delivery capabilities [18]. Several plus points make erythrocytes a promising drug delivery system. Erythrocytes are recognized as biocompatible carriers, which encourages their use in drug delivery systems [16]. Integrin-associated protein CD47 acts as a marker of self for erythrocytes. It prevents erythrocytes been destroyed by leukocytes [75]. Several other membrane parameters of erythrocytes are also well adjusted to enable structural responsivity of erythrocyte shapes (biconcave disk-like shape optimize their flow properties), thereby allowing their efficient transport to different tissues of the body [76]. Some other erythrocyte membrane composition parameters have brought about more good points in drug delivery systems. Erythrocyte glycocalyx further increases the importance of using erythrocytes in drug delivery systems. For instance, coupling tissue-type plasminogen activator (tPA) on to erythrocyte carriers has enhanced its intravascular life span and resistance to high levels of inhibitors, compared to the soluble tPA during its use for thromboprophylaxis, thanks to protection from erythrocyte glycocalyx on erythrocytes-coupled tPA [77].

Erythrocytes are the most abundantly present cell type in blood, giving an added advantage for efficient isolation of erythrocytes with low cost [73]. Also, the preparation of blood cells for drug delivery systems consists of comparatively trouble-free procedures such as density gradient separation [78]. The absence of a nucleus, mitochondria, endoplasmic reticulum in the erythrocytes of adult mammals [73] permits enough space for loading the therapeutic compound. The clearance of antibody-coated erythrocytes can also be used for specific applications. Ageing erythrocytes are usually phagocytosed by the reticuloendothelial system that allows erythrocytes as carriers for selective delivery of their payload to the reticuloendothelial system in certain disease conditions such as iron storage disease, lysosomal storage disease, and thrombocytopenic purpura [24]. In this way, erythrocyte carriers can be used to target specific destinations for a therapeutic agent. Also, a comparatively longer life span of erythrocytes can enhance the bioavailability of the therapeutic compound. Finally, it must be noted that these plus points of erythrocyte carriers

can be achieved in drug delivery advancements only by controlling therapeutic nanoparticles' material properties to limit the adverse effects to the carrier erythrocyte from them [74]. It is now clear that both partners' contribution (carrier erythrocytes and the nanotherapeutic compounds or nanocarriers loaded with a drug) is crucial for efficient and safe drug delivery to the targeted site.

7. Combined approach of natural cells and nanoparticles

As already discussed, the need for a promising solution to address the rapid clearance of nanoparticles (by the reticuloendothelial system) and other biological challenges is noticed by the scientific community. Motivated by the mammalian pathogens hemotrophic mycoplasmas that bind onto erythrocyte surface and remain in circulation for more extended periods, researchers started experimenting on combining nanoparticles with erythrocytes to neutralize the disadvantages of each partner [79]. Attachment to erythrocytes has been previously used to increase the half-lives of several drugs that can be effective while only being anchored to erythrocyte surfaces. Erythrocyte-tPA complexes (previously discussed) coupled with erythrocytes via biotin-streptavidin interactions are good examples [80, 81].

However, recent studies show the possibility of increasing the circulation time of erythrocyte-bound larger nanoparticles with a higher drug delivery payload than unbound nanoparticles. The circulation of erythrocytes was not affected by the erythrocyte-bound nanoparticles. According to the results, the erythrocytes had standard circulation times even though the nanoparticles were eventually cleared [81]. Also, the use of nanoparticles has increased the selection of drugs for effective administration. The second approach is focused on drug loading into erythrocyte carriers (unlike attaching therapeutic compounds onto erythrocytes) and show similar promising outcomes. In this case, the nanoparticles are hidden from immune cells by coating them with stealth shell (e.g., erythrocyte ghosts) [20]. In either approach, the importance of a combined system for efficient drug delivery is uncovered. Under the upcoming topics, the second approach (coating nanoparticles) will be discussed in detail.

7.1 Erythrocyte membrane-coated nanoparticles

Surface decoration is a hopeful method to enhance therapeutic agents' in vivo performance. Nanomedicine that is bioincompatible is camouflaged by coating them with erythrocyte membranes. So, our immune system will be unable to figure out the artificial (also foreign)

guest hid under the natural membrane. This concept of bridging natural and synthetic components is expected to revolutionize the field of drug delivery [19]. Based on many studies, it is clear that the erythrocyte membrane coating process is less influenced by the morphology, material type, and size of the nanoparticles. Even non-spherical particles such as gold nanocages can be successfully coated [21] in addition to typical Fe_3O_4 nanoparticles [22], and poly (lactic-co-glycolic acid)/PLGA [82]. Studies show the significance of erythrocyte membrane as a better alternative to PEG and the usefulness of CD47 markers (don't eat me marker) on the erythrocyte membrane to escape in vivo immune clearance. CD47 protein binds to inhibitory receptor signal-regulating protein, also known as SIRPα expressed on myeloid cells, and prevents erythrocytes been destroyed by leukocytes [75]. Another powerful feature of this combined system is modifying its shell and core individually [22]. This can also make us rethink modifying some important nanoplatforms less considered due to biocompatibility issues due to surface properties. One good example is carbon nanospheres serving as an optical probe and as a vehicle for therapeutic agents, but possess biocompatibility issues [83]. Novel techniques for preparing erythrocyte ghosts, drug loading, in vitro release and cellular uptake studies, and resealing erythrocytes help establish and assure this combined approach's success in the medical field [20].

7.1.1 Theoretical basis for cell membrane coating technology

Cell membrane coating technology is an active field of research applicable to nanomedicine and results in nanoparticles with properties of the nanocarriers themselves and innate cells' properties. Recent studies have revealed PEG-coated nanoparticles' rapid clearance by a phenomenon known as accelerated blood clearance. This made researchers explore novel cell membrane coating technologies [17], although using polyethylene glycol (PEG) to modify nanoparticle surfaces was once extensively employed. The preparation process of nanoparticles coated with erythrocyte-membrane (or another suitable cell membrane type) can be divided into two areas as membrane vesicle formation from erythrocytes and vesicle-particle fusion [19]. Erythrocyte membrane preparation is now realistic and simple with low-osmotic hemolysis method [84]. It has to be mentioned, even leukocyte [71], platelet [85], cancer cell [86], or other suitable cell membranes can also be used in this (Table 2).

Understanding the theoretical basis of critical concepts and procedures is crucial. It is essential to know how erythrocytes swell, and hemolyze as the salt concentration or osmotic strength of their surrounding environment decreases. This phenomenon is widely employed

in erythrocyte ghosts (dead erythrocytes without hemoglobin) preparation [87]. Inner core nanocarriers are also equally important because they act as the payloads finally delivered to the target site. Finally, the fusion of natural erythrocyte-membrane and synthetic nanoparticles can be achieved by mechanical extrusion [19] or sonication [88] (Fig 2).

Table 2. Model drugs tested in cell membrane coating technology using appropriate inner core nanocarriers.

Drug	Cell membrane type	Inner core nanocarriers	Indication	Reference
Gambogic acid	erythrocyte membrane	Poly (lactic-co-glycolic acid)	Colorectal cancer treatment	[82]
Rapamycin	erythrocyte membrane	Poly (lactic-co-glycolic acid)	Atherosclerosis	[89]
Doxorubicin	erythrocyte membrane	Mesoporous silica nanoparticles	Breast cancer	[23]
Doxorubicin	erythrocyte membrane	Magnetic nanoparticles	Ovarian cancer	[27]
Docetaxel	Platelet membrane	Poly (lactic-co-glycolic acid)	Coronary restenosis	[85]
Genistein	Macrophage	Solid lipid nanoparticles	Alzheimer's disease	[53]
5-Fluorouracil	Nanoerythrocyte membrane	Liposomes	Liver cancer	[90]
Glyburide	Neural stem cell membrane	Poly (lactic-co-glycolic acid)	Stroke	[91]
Sorafenib	U-251 MG cells membrane	Iron oxide nano-cubes	Glioma	[92]
Doxorubicin	Cancer cell membrane	Mesoporous silica nanoparticles	Prostate cancer	[93]

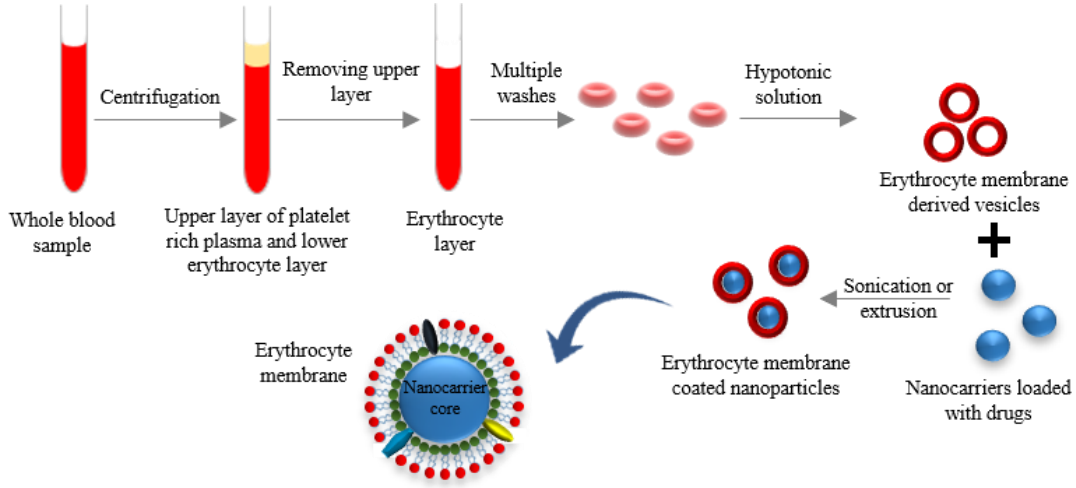

Fig 2. Preparation of erythrocyte membrane coated nanocarriers loaded with drugs. The fresh whole blood sample is centrifuged and washed to obtain erythrocytes. Then, erythrocyte vesicles (ghosts) are obtained through hypotonic treatment. Finally, nanocarriers loaded with drugs are coated with erythrocyte membrane vesicles using sonication or extrusion procedures.

7.1.2 Preparation of erythrocyte membrane-derived vesicles

The preparation of erythrocyte vesicles is an essential initial step when preparing nanoparticles coated with or loaded into carrier erythrocytes. The preparation of erythrocyte ghosts was first studied and published in 1962. As mentioned, the hemolyzing solution's pH and ionic strength affect human erythrocyte ghosts' hemoglobin content. Their procedure recovered all lipids in hemoglobin-free ghosts, and the results indicated that the lipoprotein core of prepared erythrocyte ghosts remains intact even after the hemoglobin removal [87]. Initially, a fresh blood sample, diluted with phosphate-buffered saline (PBS) at pH 7.4, can be centrifuged to purify erythrocytes [20]. As suggested, centrifugation at 2000x g for 5 minutes at 4 ºC is better [16]. Blood coagulation can be overcome by collecting fresh blood samples into heparinized containers [70]. The resulting erythrocyte pellet often has to be washed with PBS after pipetting off serum and buffy coat [16, 20]. Hemolysis (lysis of erythrocytes and release of the intracellular content) can be achieved by incubating the erythrocyte sample in 50 and 30 mOsm hypotonic solutions. The hemoglobin released into the solution can be removed by centrifugation and by washing with PBS [20]. As reported, the erythrocyte ghosts can be made effectively hemoglobin-free when prepared at pH 5.8 to 8 buffer solutions [87]. Extrusion through a 100 nm porous membrane also allows the collection of RBC membrane-derived vesicles [19]. The resulting erythrocyte ghosts are usually colourless and can be resealed by incubating in a 10x PBS (hypertonic) solution for 60 minutes at 37 ºC. After washing with PBS (isotonic) solution, the sample can be stored at 4 ºC until further use [20]. Isotonic phosphate buffer at pH 7.4 is best to obtain ghosts that retain their total lipid with significantly lower hemoglobin content [87]. In this way, erythrocyte membrane-derived vesicles (also sometimes known as erythrocyte ghosts) can be prepared with less time and effort but with high efficiency by single-stage hemolysis and washing.

7.1.3 Nanocarriers used in drug delivery

The vehicles used to store and safely deliver the therapeutic compound to the target site are nanocarriers. Inner core nanocarriers acting as ultimate payloads are equally important in this combined approach. These are usually uniform and small in size to easily accumulate in specific sites [94]. Since first described liposomes in 1964 by A.D Bangham and R.W Horne [54], several other nanocarriers with various structures are now available and are used in the medical field for drug delivery (Fig 3). They reported the results related to the structural identifications of liposomes, which later lead to the development of an efficient liposomal

drug delivery system. Nanocarriers with optimized structures are currently available as excellent candidates for drug delivery (Table 3). Only several nanocarriers are currently used in combined strategies involving cell membrane coating (Table 2). That is because the shape, size, and other nanocarriers' properties must be compatible when coating them with cell membranes. Currently, polymeric nanoparticles [19], magnetic nanoparticles [27], iron oxide nanoparticles [92], and mesoporous silica nanoparticles are successfully used in the combined strategy.

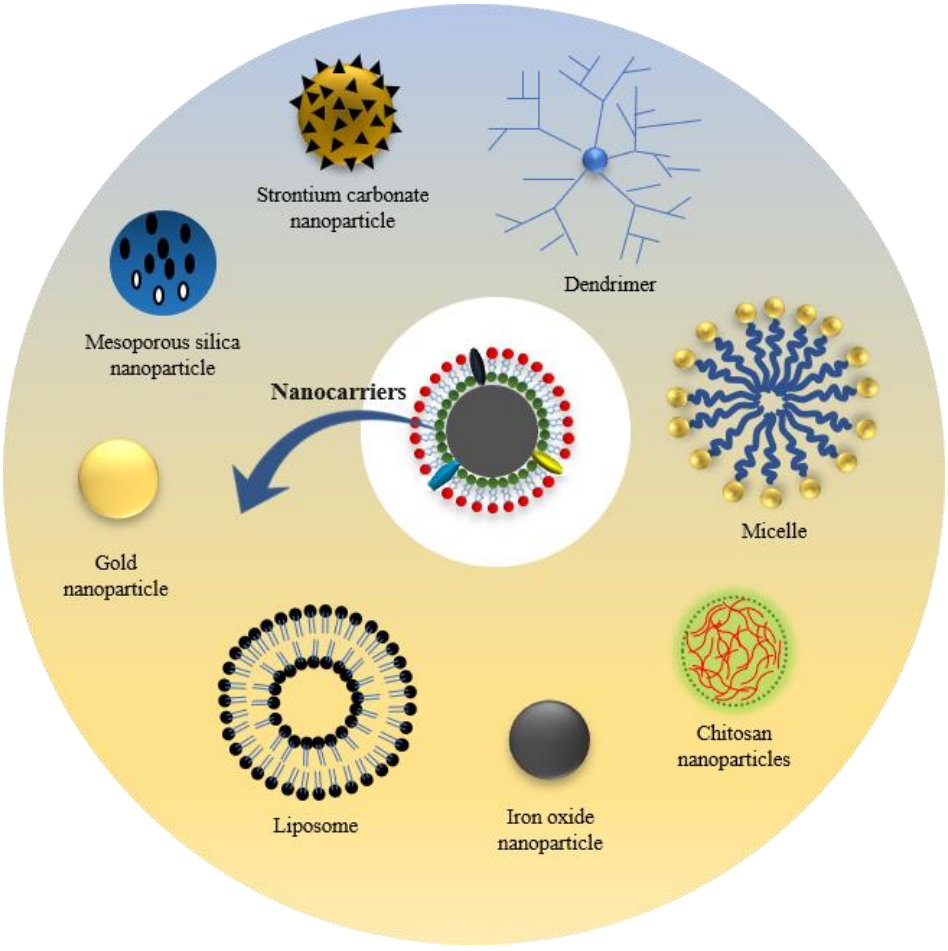

Fig 3. Different nanocarriers used as drug delivery vehicles. Nanocarriers are of various types. Some of them are polymeric nanoparticles (e.g., chitosan nanoparticles), lipid-based nanocarriers (liposomes and micelles), dendrimers, carbon nanotubes, and other inorganic nanoparticles. The drugs are encapsulated in or adsorbed on to nanocarriers. Cell membrane coated nanocarriers such as mesoporous silica nanoparticles, metallic nanoparticles, and polymeric nanoparticles are currently used in combined strategies.

Organic nanoparticles such as poly (lactic-co-glycolic acid) that is non-toxic and biodegradable are commonly used with encapsulated anticancer drugs [82]. Similarly, inorganic nanocarriers such as mesoporous silica nanoparticles with a high drug loading capacity are also widely used as an anticancer drug (e.g., doxorubicin and chlorin e6/ Ce6) delivery cargo (Table 3). The availability of well-tested procedures to produce these nanocarriers is also remarkable. Mesoporous silica nanospheres are efficiently produced

according to the Stöber method [95]. The preparation of magnetic nanoparticles to carry drugs is also well tested. Coprecipitation method using Fe^{2+} and Fe^{3+} solution containing $FeCl_2$ and $FeCl_3$ at pH 10-11 and stirring for 2 hours, followed by decantation using a magnet, can be employed. After removing excess reactants by washing with water, magnetic nanoparticles are obtained by drying in an oven [27].

Loading drugs into the nanocarriers is also relatively simple and efficient. Loading drugs into mesoporous silica nanoparticles is well-tested. In brief, the dropwise addition of the therapeutic compound solution into distilled water containing mesoporous silica nanoparticles, followed by stirring overnight to reach equilibrium. The mixture is centrifuged, followed by a washing step ensures successful loading of the drug into the nanocarriers [23]. Sometimes, drug incorporation involves an adsorption process, unlike loading the drug into the nanocarriers' core. For starch coated magnetic nanoparticles, doxorubicin can be adsorbed onto the modified nanoparticles' surface. Simple mixing of doxorubicin and nanoparticle solutions in an orbital shaker under an appropriate temperature and mixing, followed by centrifugation to collect the pellet of doxorubicin-loaded starch, and PEG diacid coated magnetic nanoparticles is sufficient [27].

Table 3. Different types of nanocarriers used as drug delivery vehicles.

Nanocarrier	Properties of nanocarrier	Possible applications	Reference
Mesoporous silica nanoparticles	High surface-volume ratio, tunable pore sizes, charge stabilized, and a surface with a large amount of Si-OH	Steady and controlled drug delivery, anti-metastasis therapies, and strategic tools for diagnosis.	[23, 95]
Gold nanocages	Hollow interior with porous walls, high absorption in near-infrared and biocompatible.	Controlled release of drugs, and in optical sensing.	[96, 97]
Strontium carbonate nanoparticles	Nontoxic, biodegradable, dumbbell-shaped morphology, high loading capacity and dense scale-like spine coating.	Controlled drug delivery, pH-sensitive drug delivery, and act as vehicles for anticancer drugs.	[94]
Chitosan nanoparticles	Linear polycationic polysaccharide, non-toxic and biodegradable.	Anti-cancer therapies, pH-sensitive delivery, and insulin delivery with high intestinal absorption in diabetes patients.	[98,99]
Dendrimers	Globular polymers, multilayered systems, improved tissue penetration, and with a polyfunctional surface.	Anti-cancer drug delivery and targeted drug delivery.	[12]
Liposomes	Bilayer vesicles formed with lipid molecules, and nontoxic.	Co-delivery of anti-cancer drugs to treat drug-resistant malignancies and pH-sensitive liposomes in endoplasmic reticulum targeted drug delivery.	[5, 45, 100,]

Iron oxide nanoparticles	Colloidal stability, biocompatibility, super magnetic characteristics and relatively low relaxivity.	Magnetic resonance imaging (MRI) and MRI visible drug delivery carriers.	[101, 102, 103]
Micelles	Spherical, made of amphipathic molecules, hydrophobic core, and low toxicity.	Anti-cancer therapies (in antitumor immunological memory), to enhance circulation time and on-off drug release: insulin loaded micelle.	[41, 104]

7.1.4 Combining membranes and particles

After preparing erythrocyte membrane-derived vesicles and inner core nanocarriers, the next step is to combine them. As already understood, this combination can involve "attachment" (therapeutic compounds attached to the membrane) or "coating" (nanoparticles coated with membranes), and this study concerns the "coating" approach. Currently, membrane extrusion is frequently used in the fusion process. This includes mixing nanoparticles with erythrocyte membrane-derived vesicles and then co-extruding through polycarbonate porous membrane using an extruder. The mixture (erythrocyte ghosts: nanoparticles) depends on the membrane volume of erythrocytes and the total membrane volume needed to fully coat a specific number of nanoparticles. The mechanical force generated by extruding facilitates the nanocarriers loaded with the drug to cross the erythrocyte membrane's lipid bilayer, resulting in the fusion of nanoparticles and erythrocyte membranes [19]. Avanti mini extruder is widely used in the coating process [27]. Another fusion method involves sonication using ultrasound energy to agitate particles in a solution. For this, a sonicator (e.g., FS3OD bath sonicator) at an appropriate frequency and power is used. In either method, successful fusion results in a proportional increase in nanoparticle diameter and an increase in surface zeta-potential [88].

8. Verification of erythrocyte membrane-coated nanoparticles

The safety, and efficiency of the combined approach are verified at several levels using accepted protocols. Some of the procedures are briefly discussed below.

8.1 Verification of surface proteins

Determination of the presence of specific proteins of erythrocyte membrane on encapsulated nanoparticles provides proof of successful coating. CD47 glycoprotein used as a marker of erythrocytes in Western blot analysis. Erythrocytes and erythrocyte vesicles will be the controls [28]. Erythrocyte membrane proteins on nanoparticles can also be characterized by treating the coated nanoparticles with lithium dodecyl sulfate lysis buffer. The sample is

denatured by heating at 85 º C for 2 minutes. Then, proteins of erythrocyte membrane coated nanoparticles can be quantified using a Bicinchoninic acid assay kit [95]. Each protein is then added into separate wells in an SDS-PAGE gel. The gel can be stained by Coomassie brilliant blue. The gel's proteins are transferred onto a polyvinylidene difluoride membrane, and Western blot analysis is done using primary antibodies specific to CD47 [95, 105]. The biological functions of erythrocyte membrane proteins expressed on the coated nanoparticles' surface can also be studied using various techniques as flow cytometry [106].

8.2 Circulation and blood retention behavior

Mice administered with fluorescently labelled, membrane coated nanoparticles through tail vein injection. Then, a small volume of blood is collected at various time intervals after the injection. Blood samples diluted with PBS can then be subjected to centrifugation, and the supernatant can be analyzed with fluorescence intensity measurements [107]. By monitoring the detectable fluorescence signal, one can get a clear idea about the blood retention time of erythrocyte membrane coated nanoparticles, compared to free drugs and uncoated nanoparticles. For example, erythrocyte membrane coated mesoporous silica nanoparticles loaded with doxorubicin and chlorin e6 have shown enhanced retention compared to the free form of a drug and uncoated mesoporous silica nanoparticles loaded with doxorubicin and chlorin e6 drugs [23].

8.3 Biodistribution

Ex vivo fluorescence imaging is used to detect the distribution of fluorescently labelled membrane coated nanocarriers injected into mice. Blood of the same mice strain is used to prepare erythrocyte membrane coated nanoparticles to avoid immune responses. The fluorescence intensity will allow the analysis of retention times of coated nanoparticles in target tissues. In oncology, deep tumor penetration is verified using the immunofluorescence technique with cryo-sectioned tumors after the injection of coated polymeric nanoparticles. Based on biodistribution study results, coated nanoparticles are frequently entrapped in tumor vessels. Co-administration of tumor-penetrating peptide iRGD with polymeric nanoparticles can increase their extravasation from tumor vessels. During histological examinations, specific markers such as proliferation indicator Ki67 can be immunohistochemically stained with green fluorescence dye to distinguish specific cancer cells in the tumor from normal cells. This allows precise observation of coated nanoparticles' biodistribution [28]. In another study, the in vivo biodistribution of primaquine was analyzed

by high-performance liquid chromatography (HPLC). The major organs of the rats administered (through the tail vein) with drug-loaded erythrocytes were excised after certain intervals from the time of injection. The organ samples were homogenized in methanol using a homogenizer, and the supernatants were analyzed for primaquine by HPLC [16]. Tests have uncovered rich distribution of coated nanoparticles in metastatic lesions suggesting that camouflaged nanoparticles with increased blood retention can ensure improved distribution within the metastatic location [28].

8.4 In vitro cytotoxicity assay

The cytotoxicity of coated nanoparticles can be examined with 3-(4,5-dimethyl thiazol-2-yl-2, 5-diphenyltetrazolium bromide (MTT) test [90]. This assay was first published by Tim Mosmann in 1983 and is a rapid colorimetric assay to detect living cells. This assay is used to measure cytotoxicity and proliferation [108]. This assay depends on reducing tetrazolium salt or MTT (yellow colour) to formazan crystals (purple colour) by metabolically active cells. This requires NAD(P)H-dependent oxidoreductase enzyme. Then, formazan crystals are dissolved, and the colour is quantified by measuring absorbance at 570 nm. The darker the solution, the higher the number of metabolically active cells [109]. In this assay, pretreated cells are seeded into the microplate wells and exposed to test material (in this case, coated nanoparticles loaded with desired drug or drugs), incubated, and used in the analysis [27, 109].

8.5 Drug release studies

The dialysis membrane bag method is used in invitro release studies [90]. Coated nanocarriers are sealed in dialysis tubing and placed into phosphate buffer (pH 7.4) in a shaking incubator at 37 º C. A small volume of buffer is withdrawn at specific time intervals and replaced by a prewarmed fresh medium. The released drug into the phosphate buffer solution is measured spectrophotometrically. The results can be compared by repeating the same procedure using the free drug in dialysis tubing. Similarly, different buffers at specific pH values can use to study drug release in various conditions [27, 110]. Dialysis tubing has a semi-permeable membrane available in multiple dimensions and molecular weight cutoffs, and selected based on the test compound. These studies have shown that nanoparticles have controlled drug release and the rate of release depends on pH, which is essential in higher release at specific sites (e.g., tumor microenvironments) [27].

8.6 Verification of linkers used to modify nanoparticles' membrane

Cell membranes used to coat nanoparticles can be adjusted using linker molecules. Cell membrane-permeable linker molecules such as succinimidyl-[(N-maleimidopropionamido)-diethyleneglycol] ester, reacting with amine groups of cell membrane proteins, can be used to conjugate thiolated molecules through thiol-maleimide reaction (Fig 4) [107, 111]. Linker length usually affects the efficiency of binding molecules onto the membrane. Using linkers with different numbers of repeating units can conjugate a targeted molecule onto the membrane [107]. For example, hyaluronidase enzyme can be conjugated, and the efficiency of conjugation can be detected using a microtiter-based assay for hyaluronidase [107, 112]. Biotinylated hyaluronan (substrate of hyaluronidase) can be coupled onto the wells of a microtiter plate. After the enzyme reaction is allowed, the substrate amount can be detected using an avidin-peroxidase reaction that can be read using an ELISA plate reader [112]. Similarly, the efficiency of membrane modification using other molecules can be analyzed using an appropriate substrate in the microtiter assay.

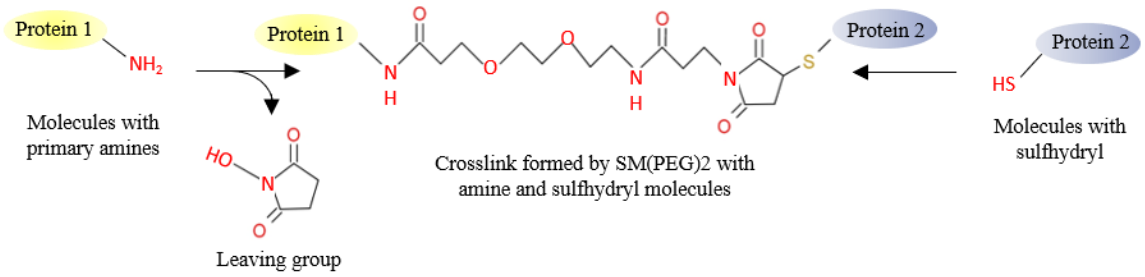

Fig 4. Thiol-maleimide reaction. Structure of crosslinks formed by the reaction between succinimidyl-[(N-maleimidopropionamido)-diethyleneglycol] ester ($SM(PEG)_2$), and amine groups (cell membrane proteins) and sulfhydryl molecules (molecule conjugated on to the membrane).

8.7 Comparison of cellular uptake of free and encapsulated drug

The efficiency of cellular uptake of the free and encapsulated drugs can be compared. For cellular uptake studies, confocal microscopy and flow cytometry can be used. In confocal microscopy, cells seeded in Petri dishes are treated with free and encapsulated drugs separately and incubated for several hours. The cells are fixed using formaldehyde, and nuclei are stained. Finally, cells are analyzed under a confocal microscope. In flow cytometry analysis, where the fixed, drug-treated cells are resuspended in PBS after collecting the cell pellet by centrifugation. Doxorubicin positive cells are then counted at λ_{EX} 488 and λ_{EM} 560 nm [113]. Cancer cells such as Hela cells can be used to test the efficiency of the delivery of encapsulated anticancer drugs such as Doxorubicin [114]. Appropriate cells can be purchased, grown in a proper medium, and monolayers of cells obtained [115]. Using these methods, studies have reported the enhanced drug uptake in encapsulated form.

9. Importance of the combined approach and it's applications

Bridging the surface properties of natural erythrocytes with the versatile drug-carrying capacity of nanoparticles has paved the way for efficient drug delivery. When considering the novel combined drug delivery system, the natural component erythrocytes help achieve drugs' targeted delivery. With erythrocytes' involvement, it is possible to target the cells of the reticuloendothelial system [24], spleen, and liver where old and damaged erythrocytes are removed [25] in a more efficient manner. Without restricting to drug delivery, erythrocytes' involvement expands the applications of the combined strategy into diagnosing diseases such as diabetes. Noninvasive, and highly sensitive paper sensor comprising polyaniline nanoparticles which are sensitive to hydrogen ions and show colour change from emeraldine blue to emeraldine green, are coated with erythrocyte membrane for this purpose [116]. Amalgamating this natural component with nanoparticles further expands the applications of the combined method. One success story is using the combined approach in ovarian cancer treatments to achieve the high anticancer activity. An excellent target for ovarian cancer treatments is folate receptors (folate binding protein) that are overexpressed in epithelial ovarian cancers [117]. For this, researchers have successfully developed folate receptor-interacting doxorubicin-loaded magnetic nanoparticles, camouflaged with erythrocyte vesicles [27].

Materials' usage to deliver therapeutic compounds overcomes the destruction of the therapeutic compound and short half-lives. It is also clear that the material usage allows constant drug release and effective targeting. But biocompatibility issues leading to rapid clearance limited the material usage in drug delivery. The drug delivery carriers mimicking erythrocytes' properties are promising candidates to address key challenges (e.g., biocompatibility and rapid clearance) faced by uncoated drug delivery carriers [18]. For instance, mesoporous silica nanoparticles with tunable pore size and high loading capacity were promising candidates for anticancer drug delivery. But limited blood circulation hindered their application. However, erythrocyte membrane coating enhanced colloidal stability and prevented the premature release of encapsulated drugs (e.g., anticancer drugs such as doxorubicin and chlorin e6) and prolonged circulation time. Additionally, using an external laser, chlorin e6 converted to reactive oxygen species in the erythrocyte membrane-coated mesoporous silica nanoparticles-Dox/Ce6, that destroy the erythrocyte membrane leading to an effective doxorubicin release [23].

Phototherapy is another area in which this combination has improved the in vivo applications. Gold nanocages showing a near-infrared absorption and high photothermal conversion efficiency are employed as photothermal conversion agents. The gold nanocages' short blood circulation time was the main drawback that limited their medical field application. But erythrocyte membrane coating has significantly enhanced in vivo retention. This allows the successful application of gold nanocages in photothermal cancer treatment. Furthermore, membrane coating has prevented the possibility of damaging major organs by these nanocarriers [21]. Bispecific targeting is also now possible with the combined strategy. Bispecific recombinant protein containing tumor penetrating peptide: internalizing RGD peptide and epidermal growth factor receptor single-domain antibody, can be inserted into the erythrocyte membrane coating. Epidermal growth factor receptor single-domain antibody targets epidermal growth factor receptor overexpressed in multiple solid tumors. In this way, erythrocyte membrane coated poly (lactic-co-glycolic acid) is modified to achieve better antitumor efficacy in colorectal cancer [82].

The significance of this combined system in toxin vaccination (based on inactivated bacterial toxins to enhance antitoxin immunity) is also reported. Higher immunogenicity and superior efficacy are achievable with this system. Safe delivery of pore-forming toxins for immune processing has become realistic at present [26]. Bacterial Infection therapies are also possible with the biomimetic drug delivery strategy. Biomimetic system carrying Tedizolid phosphate for methicillin-resistant *Staphylococcus aureus* infection is now available with high safety, and better antibacterial activity [84]. Resistant bacteria due to the antibiotics misuse, and biofilms hindering the penetration of antibiotics lowers the effectiveness of antibiotics. However, recently reported erythrocyte membrane camouflaged nanoworms capable of controlled release of antibiotics through near infrared has overcome the challenges. This nano system not only show immune escape ability inherited from erythrocytes but also effective aggregation at the infected site [118]. It is now evident that the combined drug delivery strategy showing remarkable outcomes addresses major challenges posed by nanomedicine or cell-based drug delivery alone. Prolonged circulation time, ability cross biological barriers, reduced cell toxicity, and targeted delivery of therapeutic agents are few plus points of the combined strategy [55].

10. Patents related to cell membrane coating technology

With the development of membrane coated nanocarriers, there have been a growing number of patents filed over the past few decades. Leading countries, including the United States (Table 4) and China (Table 5), have already gained several patents related to cell membrane coating technology.

Table 4. List of patents and patent applications related to cell membrane coating technology, applied by the United States (Data obtained on 10/11/2020; Google patents)

Patent or patent application publication	Title	Filing year	Current assignee
US20170143830A1	Cellular micromotors and uses thereof	2016	University of California (US)
US10117886B2	Hyaluronidase and a low density second PEG layer on the surface of therapeutic-encapsulated nanoparticles to enhance nanoparticle diffusion and circulation	2015	(US)
US20170079909A1	Hydrogel Toxin-Absorbing or Binding Nanoparticles	2015	University of California (US)
US7901674B2	Aldehyde-fixed platelets with internalized paramagnetic or magnetic nanoparticles	2007	University of North Carolina at Chapel Hill (US)
US20190382539A1	Processes and systems for preparing cellular or viral membranes and nanoparticles	2016	Arytha biosciences LLC (US)
US10434070B2	Red blood cell membranes coated nanoparticles to enable blood transfusion	2015	Cellics Therapeutics Inc (US)
US10610493B2	Detoxification Using Nanoparticles	2016	University of California (US)
US9724305B2	Nanoparticle fabrication methods, systems, and materials for fabricating artificial red blood cells	2016	University of North Carolina at Chapel Hill (US)

Table 5 List of patents related to cell membrane coating technology, applied by China (Data obtained on 10/11/2020; Google patents)

Patent number	Title	Filing year	Applicant
CN201710084446.0	Red blood cell membrane encapsulated polyester arsenic trioxide-containing nano particles and preparation method thereof	2017	Shanghai Jiaotong University (China)
CN201610148434.5	Antibody nanoparticles coated by red cell membranes for antibody drug delivery and preparation method	2016	East China Normal University (China)
CN201811003523.6	PLGA nano-carrier for erythrocyte membrane-packaged anti-cancer medicine as well as preparation and application of PLGA nano-carrier	2018	Donghua University (China)
CN201811055247.8	Preparation method of targeting and photo-thermal integrated erythrocyte bionic nanoparticles	2018	Zhejiang Sci-Tech University (China)

11. Conclusions and future perspectives

The recent awareness that drug release patterns affect therapeutic drug responses fueled the research focused on discovering new drug delivery systems. As a result, nanotechnology is

extensively applied in drug delivery to get improved therapeutic outcomes. Nanocarriers with desired biological functions and physicochemical properties treat various diseases such as cancer, central nervous system disorders, and diabetes. However, significant challenges in nanoparticles' applications are rapid phagocytic clearance, considerable degradation, and toxicity. Due to this, alternative drug delivery approaches are desirable. Extensive research has ultimately uncovered the significance of a "camouflage" comprised of natural erythrocyte vesicles and synthetic nanoparticles loaded with the drug. Erythrocytes have been widely employed in drug delivery because of their long circulating half-life, biocompatibility, biodegradability and membrane properties. This novel platform offers an opportunity to connect artificial nanoparticles' advantages with those of natural erythrocyte vesicles. Meanwhile, this combined approach neutralizes the disadvantages of the two partners. Targeted drug delivery with enhanced blood retention, safety, and efficacy are now possible with the combined strategy. The technology for preparing erythrocyte membrane coated nanoparticles is relatively mature and shows high flexibility. Therefore, it is possible to produce them on a large scale. However, the significant challenges are optimizing the fusion process and preventing contamination by erythrocyte membrane coated nanoparticles with denatured proteins. Despite a few challenges, this biomimetic nano-system provides a unique strategy and a new paradigm of thinking. Finally, this combined strategy's future trends and challenges must be addressed to transform extensive research into functional nano-biomimetic systems.

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