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Neutrophil mediated drug delivery for targeted glioblastoma therapy: A comprehensive review

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ABSTRACT

Glioblastoma is the most common brain cancer in adults and presents a major challenge for targeted drug delivery due to the blood-brain barrier (BBB) and the highly infiltrative growth of the glioma cells into the brain. Cell-mediated drug-delivery systems have been proposed as a potential strategy to enhance the effects of drugs and reduce their side effects in the treatment of cancer. Neutrophils are the most abundant type of WBC in humans and can overcome impermeable barriers and transport drugs into inflamed sites such as tumors. Therefore, a promising approach for an innovative drug delivery system is the use of neutrophils as Trojan horses for drug delivery. However, compared to other leukocytes such as macrophages, little is known about how human neutrophils respond to and take up synthetic particles. In this review, we summarize the factors affecting the uptake of nanoparticles (NPs) by neutrophils, as well as recent advances and challenges related to the interaction between neutrophils and NPs, with particular emphasis on the interaction of magnetic mesoporous silica NPs, liposomes, albumin NPs, and PLGA NPs with neutrophils. Finally, the potential application of neutrophil-based drug delivery systems for the prevention of glioblastoma recurrence and also the potential application of neutrophil-mimicking nanoparticles (NM-NPs) in glioblastoma therapy are discussed.

1. Introduction

Glioblastoma (GBM) is the most common and fatal brain tumor in adults and children, accounting for more than 65% of patients with central nervous system (CNS) tumors and is classified as astrocytoma grade IV according to the WHO classification [1,2]. Despite some success with therapies such as surgery, adjuvant radiotherapy, and temozolomide chemotherapy, there is still a high mortality rate and short median survival of approximately 12–15 months after diagnosis [3]. The primary causes of this poor survival include intratumoral and inter-tumor heterogeneity, an immunosuppressive tumor microenvironment, and tumor plasticity [4]. Current standard therapies have several challenges such as a risk of causing damage to normal tissue and unsatisfactory results. Surgery cannot prevent cancer recurrence due to the highly invasive nature of the tumor cells and their infiltration into the parenchyma of brain tissue. Moreover, tumor resection poses a potential risk for increased metastasis. Metastasis following surgical resection is a critical factor in lowering survival and quality of life in patients with GBM [5]. Chemotherapy may cause unpleasant side effects such as fatigue, nausea, infections, anemia, and other conditions caused

Abbreviations: CNS, central nervous system; GBM, Glioblastoma; DDS, drug delivery system; WBC, white blood cell; MPO, myeloperoxidase; TME, tumor microenvironment; TNF-a, Tumor necrosis factor-a; IFN-y, Interferon-Y; NPs, nanoparticles; RES, reticuloendothelial system; DOX:, doxorubicin; BSA, bovine serum albumin; PEG:, poly (ethylene glycol); PS:, poly(styrene); PLGA:, poly (lactic-co-glycolic acid); MRI, Magnetic resonance imaging; MMSNs, magnetic nanoparticles of mesoporous silica; NETs, neutrophil extracellular traps; PTX-CL /NEs, neutrophils carrying cationic liposomes loaded with paclitaxel; PBNs, peripheral blood neutrophils; PSA, Poly (sialic acid); RGD, Arg-Gly-Asp; TLRs, Toll-like receptors; PGLU, Poly-L-glutamic acid; LPS, lipopolysaccharide; PDT, photodynamic therapy; PBCA, poly(butylcyanoacrylate); OV, oncolytic virus; iPSCs, induced pluripotent stem cells; ROS, reactive oxygen species; CTCs, circulating tumor cells.

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Table 1
Neutrophils or neutrophil-mimicking NPs deliver therapeutics to different disease sites.

Disease/Side effect	NPs	Drugs	Target	Findings	References
Myelosuppression	Denatured albumin NPs	Paclitaxel	Activated neutrophils	Improved therapeutic efficacy and mitigated myelosuppression	[31]
Acute lung injury	Denatured albumin NPs	Piceatannol	Activated neutrophils	Mitigated vascular inflammation	[32]
Melanoma	Denatured BSA NPs	Pyropheophorbide-a (Ppa)	Activated neutrophils	Significantly suppressed tumor growth and increased mouse survival	[16]
Asthma	Liposomal NPs	Sodium cromoglycate	Activated neutrophils	Inhibited the influx of local neutrophil infiltration	[33]
Lewis lung carcinoma	Gold NPs decorated with Anti-CD11b antibody	NO	Activated neutrophils	Increased accumulation of anti-CD11b antibody-coated NPs in the tumor	[34]
LPS-induced lung inflammation and bacterial infection	Denatured BSA NPs	TPCA-1 and cefoperazone acid	Activated neutrophils	Dramatically alleviated acute lung inflammation/injury induced by LPS	[35]
COPD/CF lung disease	PEGylated immuno-conjugated PLGA-NPs	Ibuprofen	Activated neutrophils	Significantly delivered anti-inflammatory drug (ibuprofen) to neutrophils in murine model	[36]
Circulating CTCs, early metastatic niche, and previously formed 4T1 lung metastasis	Neutrophil-mimicking PLGA-NPs	Carfilzomib	Circulating CTCs, and early metastatic niche	Prevented early metastasis and potentially inhibited the progress of already-formed metastases	[37]
Inflamed skeletal muscle and ischemic heart disease	Liposome NPs	Methotrexate (MTX)	Inflamed endothelium	Dramatically increased the delivery of drug-loaded liposomes to inflamed target tissue	[38]

by the systemic distribution of the chemotherapeutic drugs [3].

Nanotechnology has the potential to improve cancer therapy by delivering drugs such as chemotherapeutic agents directly and precisely to cancer cells in parts of the body that would otherwise be difficult to reach, thereby improving the therapeutic efficacy [6]. However, nano-based drug delivery systems (DDS) cannot fully reach their therapeutic potential because: (1) they cannot penetrate the tumor microenvironment; (2) to avoid toxicity risks, nanoparticle (NP) doses are often kept to a minimum; (3) most importantly the ability of NPs to penetrate biological barriers, including the blood-brain barrier (BBB) is very poor [7]. Recently, nanotechnology has employed a variety of novel nanomedicine-based DDS that enable the delivery of controlled amounts of therapeutic agents into deep tumor tissues, particularly brain tumors, and has improved the survival of animal models of orthotopic glioma tumors [7,8].

The administration of therapeutics using activated neutrophils as vehicles is a promising new approach for the treatment of inflammatory diseases and cancer. Neutrophils can migrate from the bloodstream into inflamed tissue caused by infections and tumor progression. This process is very specific to the exact site of the disease. Therefore, using neutrophils as natural carriers to transport drugs or nanotherapeutics could be an innovative strategy and may enhance current treatments [9,10]. Unlike most circulating materials such as drugs and NPs, neutrophils do not rely on passive diffusion to reach the tumor microenvironment, but instead use their sophisticated cellular machinery to penetrate the tumor-associated endothelium [11,12]. While a variety of immune cells have been used as vehicles for drug delivery in models of glioblastoma, this review focuses on recent developments in the use of neutrophils for targeted drug delivery to this brain tumor.

2. Neutrophils as a drug delivery vehicle

Neutrophils are key cells participating in inflammatory situations. They account for 40–75% of circulating WBCs in human blood and are known for their ability to be recruited to sites of inflammation, where they recruit other adaptive immune cells and phagocytose foreign organisms. Neutrophils are classified as granulocytes and are characterized by their cytoplasmic granules that contain a variety of immunomodulators, proteases, and biotoxins [13]. There are several subtypes of neutrophil granules that are functionally and structurally different, each released in response to specific stimuli. These include primary or azurophilic granules [containing myeloperoxidase (MPO)], secondary or specific granules (containing lactoferrin), and tertiary or gelatinase granules (containing gelatinase) [14,15]. Neutrophils can be manipulated to stimulate the release of a specific granule for therapeutic purposes, or synthetic NPs can be introduced into neutrophils and released at the site of inflammation. The latter approach is a fascinating new idea in the field of immune engineering [16]. Neutrophil-mediated drug delivery systems are based on dysregulated inflammatory stimuli originating from the tumor mass or present in inflammatory/autoimmune diseases. In the tumor microenvironment, cytokines and chemokines are produced by both tumor cells and stromal cells, forming a particularly rich "soil" that facilitates the infiltration of various immune cells into the tumor microenvironment (TME)[17,18]. Neutrophils are an important component of the immune cells that infiltrate the TME. In GBM, increased numbers of infiltrating neutrophils are associated with high-grade disease and are considered a sign of tumor progression, invasiveness, and acquired resistance to anti-VEGF treatment. Tumor-associated neutrophils (TAN) exist in two polarized states within the glioma microenvironment (GME): N1 anti-tumorigenic phenotype induced by IFN-β stimulation; N2 pro-tumorigenic phenotype induced by TGF-β and G-CSF stimulation. Primary mediators controlling the selective recruitment of neutrophils to the TME include tumor necrosis factor α (TNF-α), interferon-γ (IFN-γ), IL-8, and IL-1 [19].

Nanotechnology is now being broadly used in a range of cancer treatments. Currently approved NPs for cancer treatment include liposomes [20], polymeric NPs [21], dendrimers [22], and magnetic NPs [23] designed to improve targeting and increase the local therapeutic efficacy. However, most intravenously administered NPs often do not successfully reach the tumor mass due to biological barriers, serum instability, high clearance rate, and immunogenicity. These challenges have limited their utility in the treatment of inflammation and cancer [24].

Recently, researchers have focused on employing immune cells as natural vehicles to actively deliver NPs containing high concentrations of a drug across blood vessel barriers. Because therapeutic NPs are nanoscale in size, they are ideal for immune cell targeting, binding, and uptake[25]. Neutrophils are the ideal immune cells for transporting NPs because: (1) they are the first type of leukocyte attracted to the tumor in response to different chemokines released after surgical resection [26]; (2) they are the most abundant leukocytes during acute inflammation compared to monocytes and lymphocytes; (3) they have the potential to attract additional circulating neutrophils [25,27]. The vast majority of drugs cannot be administered directly since they are cytotoxic to

neutrophils. Since NP internalization does not affect neutrophil survival, apoptosis, or activation, neutrophils can be loaded with NP-drug complexes to provide an effective solution to these challenges. Previous studies have shown that neutrophils loaded with NP-drug complexes were not affected by the drug at a therapeutic dose and maintained their physical structure, biological function, and migration in response to inflammatory triggers[28,29]. Interestingly, some of the disadvantages of nanotechnology-based DDS, such as poor biocompatibility, difficulty in transmigration across the BBB and other biological barriers, non-specific targeting, and harmful side effects to the recipient, can be addressed by neutrophil-based DDS [30]. Several intriguing studies have suggested that drug delivery by neutrophils has the potential for the treatment of inflammation and cancer. There is evidence that neutrophils loaded with therapeutic NPs can infiltrate into the tumor center. However, due to the unique constraints of clinical trials, relatively few human studies have been conducted to confirm the clinical effectiveness of this method[27]. Table 1 summarizes the current state of neutrophil-mediated DDS for delivering treatments to different disease sites.

In addition to the toxicity of chemotherapeutic agents to living cell carriers, the direct use of neutrophils as vehicles for drug delivery presents several other challenges, such as the short lifespan (half-life 1–5 days) of neutrophils, which necessitates the isolation, purification, drug loading, and reinfusion of drug-loaded neutrophils within a few hours. Furthermore, rapid intracellular degradation of the cargo, high cost, and insufficient volume of harvested cells are other challenges [39,40].

In general, neutrophil-NP complexes can be prepared by one of two methods. In one method, the NPs are taken up by neutrophils in vitro before being injected into the bodies of animals. In the other method, the nanomaterials are taken up in vivo by circulating neutrophils after injection into the bloodstream [35,41]. The second method, in which free nanomaterials are injected into the body, presents a number of difficulties, including clearance through the RES (reticuloendothelial system) and the need to rationally design nanomaterials with high binding affinity and specificity for neutrophils [42].

Due to the aggressive nature of glioma tumors and the infiltration of cancer cells into the normal parenchyma of the brain, the tumor mass cannot be completely removed from the healthy brain by surgery because of the risk of damaging healthy brain tissue [5]. Furthermore, subsequent chemotherapy is unable to deliver a sufficient dose of chemotherapeutic agents to eliminate the remaining cancer cells due to the difficulty of crossing the BBB, while the systemic distribution of chemotherapeutic agents in the body limits the total dose that can be safely administered [43,44]. Interestingly, Xue et al. reported that neutrophil-mediated drug delivery significantly inhibited tumor recurrence in mice with surgically operated GBM tumors [18]. They encapsulated paclitaxel (PTX), a major chemotherapeutic drug used to treat glioma, into cationic liposomes (PTX-CL), which were subsequently internalized by neutrophils to form NE-based delivery vehicles (PTX-CL/NEs). In operated mice with GBM, intravenous administration of PTX-CL/NEs led to neutrophil accumulation in the tumor site under a gradient of inflammatory signals (TNFα and IL8) released from cancer cells, resulting in the release of PTX into the remaining cancer cells, significantly prevented tumor recurrence and led to complete recovery of the mice [41,45,46]. Other studies have also shown that neutrophils carrying cationic liposomes loaded with coumarin-6 (Cou6) (Cou6-CL/NEs) could dramatically increase survival and reduce tumor recurrence in tumor-bearing mice by invading the tumor microenvironment[18,47].

Thus, when the tumor is inflamed or surgery is performed, an increase in inflammatory cytokines is a prerequisite (and not a disadvantage or advantage) for using neutrophils as a vehicle for drug delivery. In contrast to GBM, where neutrophil-mediated drug delivery should follow surgery (post-surgery GBM), neutrophil-mediated drug delivery in inflammation-related cancers such as colorectal cancer in inflammatory bowel disease (IBD) may be possible without surgery. In the future, acute inflammation in tumor tissue triggered by photodynamic therapy using photosensitizers may be one alternative to surgery to allow neutrophil-mediated drug delivery [34]. Photodynamic therapy is the process by which a photosensitizer absorbs a specific wavelength of light to reach a higher energy state, and then transfers this energy to molecular oxygen, which is then converted into a variety of reactive oxygen species (ROS). ROS can cause acute inflammation, which allows neutrophils to invade the tumor by rapid activation [34,48].

The use of leukocytes for drug delivery is not limited to the prevention of recurrence, but can also be used to deliver therapeutic drugs to metastatic circulating tumor cells (CTCs). GBM is the most aggressive intracranial neoplasm and usually invades into adjacent brain tissue, but rarely migrates to distant tissues such as the pleura or lung, with an incidence of only 0.2%. Nevertheless, delivering therapeutics to metastatic circulating tumor cells (CTCs) to prevent metastasis has been a challenge for many years. This is because their concentration (average 8 cells/mL) in the bloodstream is very low [49,50]. Interestingly, research has shown that CTCs can actively bind to leukocytes through their adhesion molecules. This property can be used to target circulating tumor cells with neutrophil-loaded drugs to prevent metastasis [50]. Recently, neutrophils were found to bind directly to CTCs via two important proteins, VCAM1 (Vascular Cell Adhesion Molecule 1) and ICAM-1 (Intercellular Adhesion Molecule 1). ICAM-1 promoted liver metastasis by facilitating interactions between CTCs and liver endothelial cells, while VCAM1 enhanced binding between neutrophils and CTCs in the circulation[51–53].

Inspired by the interaction of neutrophils with CTCs, researchers developed a nanoscale neutrophil-mimicking drug delivery system (NM-NP) by coating the membranes of neutrophils onto the surface of NPs. NM-NPs can specifically increase the inhibition of CTCs in the circulation, prevent early metastasis, and possibly reduce the development of already formed metastases[54]. There are numerous obstacles to the direct use of neutrophils as therapeutic drug carriers, such as the difficulty of in vitro culture, because neutrophils are terminally differentiated cells with a half-life of only 7 h. Recently, the use of biomimetic nanotechnology for drug carrier production has received much attention and may provide a potential solution to the problem of the direct use of neutrophils. NM-NPs are more stable and targeted compared to conventional NPs and are more likely to concentrate in the tumor microenvironment [55]. For example, Chen et al. designed a nano-sized NM-NP by coating inflammatory neutrophil-derived membranes onto the surface of PLGA NPs to reduce metastatic burden by targeting CTCs both in the circulation and in the premetastatic niche. The NPs were approximately 100 nm in diameter, and membrane-associated protein complexes on neutrophil membranes were transferred to the surface of NM-NP using a non-destructive approach. NM-NPs were termed "super neutrophils" because the biological binding activity of neutrophils was hardly affected. To inhibit metastasis, they loaded the NM-NPs with carfilzomib (CFZ) a second-generation selective proteasome inhibitor. The resulting NM-NP-CFZ showed a stronger cellular interaction with CTC under shear stress in vitro, significantly increased CTC trapping efficiency in vivo, and specifically homed to the premetastatic endothelium. NM-NP-CFZ significantly reduced CTCs in blood, and had the therapeutic potential to both prevent new metastases and inhibit existing metastases [37,56]. For NM-NPs to interact with cancer cells, membrane-associated protein complexes must be fully available on the surface of PLGA NPs. To achieve efficient drug delivery, NM-NPs should contain functional proteins, such as selectins, α2, β1-integrins, and CXCR4, which together act as mediators between NM-NPs and the inflamed endothelium [37,57]. While NM-NPs have been used for tumor targeting, their antitumor effects are still uncertain due to questionable uptake by cancer cells.

In several studies, NM-NPs have been modified by adding different ligands to induce receptor-mediated endocytosis to promote their internalization. Glioma-specific ligands, including TRAIL, anti-EGFRvIII, OX26, anti-TfR, and anti-CD133 have been used to promote

the internalization of NM-NPs by inducing receptor-mediated endocytosis by glioma cells. These ligands can respectively bind to the overexpressed receptors on glioma cells, such as DR4, EGFRvIII, transferrin, and CD − 133 [58–61].

3. Factors Affecting NP Uptake by Neutrophils

Nanoparticle uptake by neutrophils depends on several parameters, including the size and shape of the particles, concentration, type of material (liposomes, gold, albumin), surface chemistry, and the presence/absence of a serum protein corona. Knowing how these factors affect the interaction of NPs with neutrophils is critical for advances in NP-based therapeutics and improved cell targeting [62]. The size of particles affects how well they are absorbed by cells. Studies on size-dependent uptake of NPs have shown that smaller NPs (about 30–50 nm) are more readily taken up by receptor-mediated endocytosis in tumor cells [62]. By contrast, particles ranging in size from 20 nm to 200 nm are more effectively taken up by neutrophils compared to smaller particles. This is an intriguing finding that differs from the common notion that smaller NPs are more suitable for tumor therapy due to the EPR effect (enhanced permeability and retention) [28,63]. Overall, studies have shown that particles ranging in size from 20 nm to 1 μm are rapidly internalized by human neutrophils *ex vivo* at concentrations up to 0.5 mg/mL [28,39]. One of the most important variables for cellular uptake is the shape of NPs. It has been observed that NPs less than 5 nm in diameter are rapidly taken up by cells regardless of their shape. In contrast, for larger NPs, the shape is important for cell uptake. It has been proposed that spherical NPs are better absorbed by cell membranes than rod-shaped NPs [64,65], however, whether all spherical NPs are better absorbed than rod-shaped NPs is still controversial.

Surface chemistry, along with size and shape, significantly affects the intracellular uptake of particles. Although both aspect ratio and surface chemistry affect cellular internalization, only surface chemistry affects cytotoxicity [66]. Surface chemistry can precisely regulate and modify the surface properties of NPs to meet the requirements of applications ranging from diagnostics to therapeutics [28,67]. Since the cell membrane and many NPs both carry anionic charges under physiological conditions, NPs with a positively charged surface generally show better binding with the anionic cell membrane and also higher internalization rates, and are therefore better absorbed than negatively charged NPs [66,68]. For example, coating the surface of NPs with the cationic lipid stearylamine is a common approach to enhance the uptake of negatively charged PLA-PEG NPs by neutrophils [69].

Regarding NP surface functionalization, Zhang et al. have developed NPs containing doxorubicin (DOX) coupled to bovine serum albumin (BSA) via hydrazone bonds to target activated neutrophils in vivo. DOX-loaded NPs have been used to treat two disease models (cerebral ischemia/reperfusion and sepsis) to increase intracellular transport of DOX into neutrophils and induce neutrophil cell death. DOX-hyd-BSA NPs were administered intravenously and specifically targeted active neutrophils in the circulation, decreased neutrophil transmigration, and attenuated inflammatory responses. Their results suggest that NPs targeted to active neutrophils may be a potential treatment option for inflammatory diseases associated with neutrophil overactivation [29,70]. In another study, liposomes conjugated with poly(ethylene glycol) PEG were found to be more efficiently absorbed by human neutrophils in whole blood compated to liposomes without PEG[69,71,72]. In addition, surface modification of NPs with PEG decreased phagocyte clearance, nonspecific adhesion and absorption, and increased the circulation half-life of the particles in vivo. Several PEGylated particle compositions have been approved by the FDA to provide an extended half-life in the circulation[73], although these compositions are liposomes rather than polymers [69,72].

Interestingly, NPs can absorb varying amounts of serum proteins on the particle surface as they travel through the bloodstream, which is referred to as the protein corona. The NP protein corona is referred to as the "true identity" of NPs in the human body. The uptake of serum proteins is influenced by the surface properties of NPs as they modulate protein binding [74,75]. In addition, other physicochemical properties of NPsm such as electrostatic charge, nanomaterial type, surface chemistry, and shape can also have a significant impact [75]. The proteins most commonly adsorbed by NPs include albumin (most abundant), complement proteins, fibrinogen, and immunoglobulins. It is noteworthy that the composition and relative amount of adsorbed protein corona is unique for each nanomaterial and NP type [76]. Bisso et al. studied the effects of physiologically relevant amounts of serum proteins bound to NPs on uptake by neutrophils, and found that the presence of serum proteins increased the uptake of certain NPs by neutrophils compared to naked NPs. The presence of human serum protein decreased the uptake of certain particles, such as poly(styrene) (PS) and liposomal NPs, while it increased the uptake of other NPs, such as poly(lactic-co-glycolic acid) (PLGA) NPs [69,77,78].

The decrease in the absorption of NPs in the presence of serum proteins could have three possible explanations: (i) exposure to serum leads to agglomeration of nanoparticles; (ii) masking of the particle surface by serum proteins creates a barrier to the "sticky" interactions between the hydrophobic surface of NP and the membrane of a neutrophil by affecting the size and surface charge; (iii) neutrophils exposed to protein-coated particles in serum are less likely to take up other particles [79]. Particles that can be internalized by neutrophils and could be used for drug delivery include liposomes, PS (polystyrene), PLGA (poly D, L-lactic-co-glycolic acid), magnetic mesoporous silica NPs, gold NPs, and albumin NPs. In the following section, we summarize the properties of some of these NPs and their interactions with neutrophils in animal models.

4. Common NPs investigated for neutrophil-mediated drug delivery

4.1. Magnetic mesoporous silica nanoparticles

Neutrophil-mediated drug delivery systems are potentially suitable for targeted tumor therapy, because neutrophils show inherent tumor-homing and drug-carrying properties. However, improved methods for imaging and tracking neutrophil migration into the tumor sites are urgently needed before any clinical usage, particularly for glioma therapy. Magnetic resonance imaging (MRI) is a unique tool for in vivo imaging and tracking of cells in the brain because it is noninvasive, highly sensitive, and allows relatively long-term tracking of MRI contrast agents introduced into transplanted cells [80]. To identify cells of interest using MRI, the cells must be labeled to produce a strong contrast. On the other hand, the integration of MRI contrast agents directly into neutrophils may lead to a variety of physiological changes. It should be mentioned that neutrophils are known to be able to take up a wide range of NPs. A research team led by Zheng et al. recently developed an innovative biocompatible therapeutic and diagnostic approach for drug delivery into inflammatory glioma tumors after surgical resection [81,82]. They allowed activated neutrophils to take up magnetic mesoporous silica NPs (MMSNs) in vitro to improve neutrophil monitoring by MRI after injection into mice. MMSNs are core-shell structures that can act as both neutrophil tracking probes and nanocarriers for drug delivery. In mouse models, this approach greatly enhanced intratumoral drug accumulation and delayed tumor recurrence [81,82]. The MMSN complex usually consists of a magnetic Fe3O4 core as a contrast enhancer for MR imaging, and a mesoporous silica shell that serves as a drug carrier due to its large surface area and adjustable pore size [83,84].

Recently, MMSNs have been investigated for a variety of clinical applications, such as drug/gene delivery, delivery of active forms of proteins, diagnosis, cell uptake, and laboratory separation procedures [83,85]. In drug delivery, MMSNs show a good ability to reach tumors and have useful properties such as large surface area, large pore size, and easy surface modification [86,87]. Studies have shown that MMSNs

have high cellular uptake and stability within neutrophils, with no obvious cytotoxic effects, implying that MMSNs have good biocompatibility in neutrophils [88]. MMSNs have a significant drug loading capacity, and after injection of neutrophils loaded with MMSNs, they can be tracked by MRI to actively target the inflamed tumor region after surgical removal of the primary tumor to limit cancer recurrence [88]. Numerous studies have reported that this strategy significantly improved intratumoral drug concentrations and increased survival in mice with surgically removed GBM tumors. Zheng et al. demonstrated that neutrophils carrying MMSNs loaded with the anticancer drug DOX (known as ND-MMSNs) retained their biological function and migrated toward inflammatory brain tumors, resulting in longer survival of glioma-bearing mice after surgical tumor removal [35,88,89]. After surgical resection of the main tumor, the ND-MMSNs were injected intravenously into a mouse model with residual inflammatory glioma, where they encountered a chemokine gradient that led to their activation and migration to the site of inflammation [90]. The ND-MMSNs accumulated in the inflamed glioma, resulting in increased neutrophil recruitment and prolonged residence time [91]. In this context, highly active ND-MMSNs can induce the formation of neutrophil extracellular traps (NETs) in the inflammatory focus, thereby scavenging pathogens and releasing neutrophil granules. Glioma cells absorb the released neutrophil granules, resulting in enhanced MRI imaging and potent anti-glioma activity [87,92,93]. The ND-MMSNs had a dual purpose in that they could transport doxorubicin across the BBB while also allowing in vivo MRI monitoring of the cells due to the magnetic Fe3O4 core, which enhanced MRI contrast [91].

4.2. Liposomes

Liposomes are nanoscale or microscale vesicles consisting of one or more lipid bilayers surrounding an aqueous core. Liposomes are used as a therapeutic drug delivery system due to their low systemic toxicity, biodegradability, adaptable physicochemical properties, ability to carry large amounts of drugs, and extensive pharmacological inactivity. Moreover, liposomes can entrap molecules with varying degrees of lipophilicity due to their inherent structure [20,94]. However, the use of liposomes as drug delivery vehicles is associated with several challenges, including RES sequestration of liposomes from the bloodstream, destabilization of liposomes in the bloodstream by interaction with plasma proteins, triggering of innate immune responses, and in the case of glioblastoma, the inability to cross the BBB efficiently [95]. Researchers have shown that neutrophils loaded with liposomes containing chemotherapeutic drugs can not only overcome the limitations of liposomes in drug delivery, but also provide additional advantages, such as rapid penetration of the BBB and improved targeting of the tumor [95,96]. For example, Zhang et al. showed that neutrophils loaded with cationic liposomes containing paclitaxel (PTX-CL /NEs) significantly inhibited the recurrence of glioma tumors in mice whose tumor mass had been surgically removed, and improved the survival rate of the mice by nearly 50% [41,97]. Intravenously administered PTX-CL/NEs can respond to postoperative proinflammatory cytokines such as IL − 8 and CXCL1/ KC and migrate along the chemoattractant gradient to reach the remaining glioma cells [98]. A highly concentrated inflammatory signal generated by the remaining glioma cells induced neutrophils to release PTX-loaded liposomes by forming NETs [18,97]. PTX-CL could efficiently deliver PTX into tumor cells and showed higher cytotoxicity to prevent tumor recurrence. However, no significant anti-tumor effects were observed in mice with primary glioma tumors, suggesting that the increase in inflammatory signaling produced after surgical resection helped to improve brain tumor targeting and the clinical effects of PTX-CL/NEs [41]. Although the study proved the excellent migration and therapeutic efficacy of neutrophil-mediated PTX-CL drug delivery in vitro and in vivo, the system required an additional step of neutrophil transfusion, which can severely damage neutrophils. Therefore, direct delivery of the drug to activated neutrophils in the circulatory system might be a better solution. In a separate study, Luo et al. observed that liposomes coated with polysialic acid (PSA) enhanced the absorption of liposomes by neutrophils in vivo, resulting in a higher anticancer effect than the formulation PTX-CL [99]. PSA is a non-immunogenic biopolymer composed of α2,8-linked sialic acid moieties that can target peripheral blood neutrophils (PBNs) via recognition of L-selectin [100]. Upon injection of PSA-decorated liposomes into the bloodstream, they efficiently target neutrophils, which are then attracted to the TME by inflammatory chemokines [101].

Studies have shown that liposomes can undergo other types of surface functionalization to increase their binding to monocytes and neutrophils [102]. Neutrophils express heterodimeric proteins on their surface consisting of combinations of α- and β-subunits called integrin receptors. These receptors selectively bind to the tripeptide Arg-Gly-Asp (RGD) motif contained in their ligands [103]. The RGD domain binds to the integrin receptor on neutrophils and induces them to undergo phagocytosis. Due to their ability to undergo phagocytosis, diapedesis, and chemotaxis, neutrophils can be used as a delivery system for selective and preferential drug delivery [104,105]. Recently, RGD-coated liposomes were found to be preferentially taken up by circulating neutrophils via facilitated receptor-mediated endocytosis. In this way, liposomes can be taken up into the brain in response to inflammatory cell recruitment[105], providing an innovative strategy for brain-targeted drug delivery [102,106,107]. In addition to integrin receptors, mannose receptors, Toll-like receptors (TLRs), L-selectin, complement and Fc receptors on the neutrophil membrane may also be involved in the recognition and uptake of NPs. Mannose receptors interact with mannan residues on NPs, while complement receptors recognize NPs and form nonspecific NP-antibody complexes, and FC receptors interact with NPs to form specific NP-antibody complexes [108,109]. Furthermore, positively-charged NPs such as polyethylenimine (PEI) and poly-L-lysine (PL) are more likely to be taken up by neutrophils than neutral NPs, or negatively-charged NPs such as poly-L-glutamic acid (PGLU) (about twofold higher) [108,109].

4.3. Albumin nanoparticle hijacking of neutrophils

The serum protein albumin has been used to produce albumin-based nanocarriers because it is biodegradable, nontoxic, and non-immunogenic. Studies have shown that intravenously (iv) infused albumin NP is specifically internalized by activated neutrophils (under inflammatory conditions) but not by resting neutrophils, after which neutrophils containing albumin NPs are transported across the blood vessel barrier and accumulate at inflammatory sites caused by pathogens or cancer invasion [35]. Moreover, the uptake of albumin NP did not affect cytokine production, neutrophil mobility, or activation [110, 111]. Wang et al. demonstrated that neutrophils bearing albumin NPs composed of BSA coupled with a powerful, specific inhibitor of IB kinase (TPCA-1) had a substantial therapeutic effect with reduced lung permeability in acute lung inflammation or injury compared with other therapeutic formulations [111]. In mouse models, infusion of albumin NPs loaded with an anti-inflammatory drug followed by absorption by activated neutrophils (but not resting neutrophils) substantially decreased LPS (lipopolysaccharide) or *Pseudomonas aeruginosa*-induced lung inflammation. This was most likely related to a decrease in the neutrophil count and the concentration of proinflammatory cytokines IL − 6 and TNF-α in the lungs [112,113]. According to the findings, neutrophils could be used as carriers to actively deliver drug-loaded albumin NPs to sites of inflammation and cancer.

The uptake of albumin NP by neutrophils is selective and is mediated by neutrophil receptors, such as the Fc gamma receptor III (Fc gamma RIII; CD16). Fc gamma RIII is abundantly expressed on activated neutrophils and is required for the uptake of albumin NPs by neutrophils [114]. Blocking these receptors in genetically altered mouse models significantly impaired the uptake of albumin NPs by neutrophils compared with WT (wild-type) mice [35,115]. Since activated

neutrophils play an essential role in inflammatory diseases and cancer, targeting these activated neutrophils represents an innovative strategy for the treatment of inflammation and cancer [116]. The use of albumin NPs to target activated neutrophils may be a promising strategy for the treatment of chronic inflammatory diseases and tumors characterized by neutrophil migration and vascular adhesion. It is worth noting that increased neutrophil infiltration in cancer is associated with tumor progression and metastasis [116,117]. Therefore, targeting and utilizing active neutrophils may be a promising strategy for cancer treatment if the tumor progression is related to neutrophil recruitment and vascular adhesion. Previous studies have shown that TA99 monoclonal antibodies can promote neutrophil recruitment via a mechanism of antibody-dependent cellular cytotoxicity (ADCC). Consistent with this finding, Chu et al. developed a technique combining TA99 and albumin NPs to treat cancer in a mouse model [116,118]. Chu et al. reported that injection of TA99 (a monoclonal antibody) into the bloodstream of mice with melanoma tumors increased the migration and activation of neutrophils loaded with albumin NPs containing a photodynamic therapeutic agent (pheophorbide a, PPa) into the tumor, compared with a control group without administration of TA99 [116,119–121]. Photodynamic therapy (PDT) significantly reduced tumor development and enhanced lifespan when compared to PPa-NPs or TA99 therapy alone [121]. In addition, neutrophils took up significantly more albumin NPs after TA99 injection. The results showed that the simultaneous administration of TA99/PPa-NPs to mice implanted with melanoma followed by light irradiation reduced tumor development and significantly prolonged the survival time of the mice, suggesting a unique approach to cancer therapy that combines NPs targeting immune cells with ADCC [116,122].

4.4. PLGA-based nanoparticles and neutrophils in cancer treatment

Various synthetic or natural polymers have been used for targeted drug delivery. Natural polymers include chitosan, alginate, hyaluronic acid, elastin, and albumin (83). Polybutylcyanoacrylate (PBCA), poly D, L-lactic-co-glycolic acid (PLGA), acrylic polymers, poloxamers, and polaxamines are all examples of synthetic polymers used for drug delivery. Some synthetic polymers have higher biodegradability and biocompatibility than certain natural polymers, which may be beneficial in clinical studies [123,124].

PLGA has recently emerged as the most well-known and widely used material to prepare nanoparticles for the active delivery of antitumor drugs to the tumor microenvironment. Due to its flexibility, simple formulation, easy biodegradability, and capability to regulate the release of various cargoes, PLGA has attracted preclinical and clinical interest. Hua Tang et al. found that neutrophils (but not other leukocytes) preferentially internalized PLGA NPs with a diameter of 260 nm [125,126]. They reported that in mouse models, the combination of PTX-PLGA NPs with the preimplantation of biocompatible, thermosensitive CXCL1-loaded hydrogels in tumor sites resulted in better tumor suppression than PTX-PLGA alone [125,127]. The continuous release of CXCL1 from the hydrogel created gradients that attracted neutrophils with payloads and concentrated them at the implanted site [111,125]. Therefore, neutrophils can act as transporters of therapeutic PLGA nanoparticles to inaccessible tumor sites due to their chemotactic effect [128]. They demonstrated that PLGA NPs had little effect on neutrophil normal physiological function and caused minimal damage to neutrophils, even after prolonged exposure to PTX-PLGA NPs. As a result, neutrophils can be considered excellent transporters for therapeutic PLGA nanoparticle delivery in vivo. The therapeutic efficacy in treated mice was very high, with the strongest tumor suppression (67.28%) compared to liposomes containing PTX (31.44%) and PTX-PLGA NPs without hydrogel (46.95%) [125]. Other studies have shown that serum proteins such as BSA are required for efficient internalization of PLGA nanoparticles by neutrophils, with approximately 6-fold higher internalization compared to uncoated NPs [129]. In addition, PLGA NPs coated with native BSA have many advantages over BSA-free NPs, such as the ability to load more different drugs with simple procedures, and to adjust the drug release rate depending on the application [129].

5. Neutrophil-mediated viral delivery

Gene therapy requires the ability to transduce therapeutic plasmid DNA, mRNA, or siRNA into non-dividing cells for sustained in vivo gene delivery. Transduction and transfection are the methods for introducing genetic material into host cells in vitro and in vivo. Transfection can involve non-viral methods to create temporary holes in the cell membrane through which nucleic acids can enter. Transduction is a typical technique for introducing DNA or RNA into host cells using viral vector carriers.

Cell-mediated viral delivery to tumors has now received a lot of interest. Systemic administration of oncolytic viruses (OV) is usually unsatisfactory, because the viruses are eliminated by host immune defense systems, taken up by other organs, or nonspecifically targeted to normal cells. These problems result in the poor clinical activity of oncolytic viruses after systemic administration. It is well known that most cancer microenvironments are heavily infiltrated by various leukocytes in both primary and metastatic tumors. Therefore, immune cell-mediated delivery of viruses for tumor eradication may be a promising therapeutic approach. Based on virus-mediated DNA transfer, in vitro transduction can efficiently deliver OVs into circulating cells to target a tumor while protecting the virus from elimination by host defenses. Adenoviruses, herpes simplex virus, vaccinia virus, myxoma virus (MYXV), vesicular stomatitis virus, measles virus, retroviruses, influenza virus, and reovirus have all been tested with various cell-based delivery systems. These oncolytic viruses can be based on naturally occurring viruses that specifically kill tumor cells, or on viruses that have been genetically engineered to attack tumor cells. Numerous studies have shown that activated human leukocytes exposed to OVs can transfer the viruses to cancer cells by heterologous cell fusion. This approach has been shown to cause cancer cell elimination and increase survival in mouse tumor models [130,131]. T lymphocytes, cytokine-induced killer (CIK) cells, and neutrophils have all been investigated as potential carriers of oncolytic viruses to tumors. However, little is known about the interactions between oncolytic viruses and neutrophils. Lilly et al. found that neutrophil populations isolated from bone marrow transplants (BM) could act as effective MYXV-armed carrier cells to attack cancer cells in the bone marrow or spleen [132]. They demonstrated that ex vivo MYXV-loaded neutrophils successfully delivered MYXV to cancer cells, and killed residual myeloma cells in vitro. Neutrophils carrying MYXV can also interact with and activate natural killer (NK) cells, dendritic cells (DCs), macrophages, as well as T and B cells by releasing a variety of cytokines and chemokines. This was the first study to show that neutrophils could be a viable viral carrier for oncolytic virotherapy [132]. However, future in vivo research will be required to confirm the role of neutrophils suggested by the in vitro results. Interestingly, the viral replication cycle in T cells infected with measles virus appeared to be aborted, even when the T cells had been preactivated. This was shown because they expressed GFP encoded by the virus, but did not develop characteristic cytopathic effects or release infectious virions. Although they did not produce free virions, infected T cells were still able to transfer measles virus infection to tumor cells by a cell-cell fusion mechanism. Theoretically, the non-replication of virus could still support viral transmission by giving infected T cells sufficient time to reach the tumor before releasing their oncolytic payload and compromising T cell survival[133]. In addition to using immune cells as vehicles for OV transfer, mesenchymal stem cells (MSCs) have also been investigated for OV transfer with promising results [134,135]. For example, Hu et al. demonstrated that transfection of tumor necrosis factor related apoptosis-inducing ligands (TRAILs) into MSCs using a nonviral vector resulted in significant anticancer effects in a lung metastasis model [136]. However, this technique is still in its infancy with respect to

glioblastoma, and further study is needed to confirm its safety for future clinical applications.

6. Uptake of drugs/nanoparticles by neutrophils

The efficacy of neutrophil-mediated drug delivery to tumors is highly dependent on the loading capacity of the cells. Most studies have focused on the cytotoxic effects of interactions between neutrophils and NPs, whereas the molecular mechanisms of NP uptake by neutrophils are still unknown. The processes of internalization of NPs are well understood in monocytes and macrophages, however it is difficult to extend these results to neutrophils due to the difficulty of genetic manipulation of neutrophils. To achieve cell-mediated drug delivery, drugs or drug-loaded NPs must be able to bind to or be absorbed by the cells to form drug-cell or particle-cell complexes. Particle-loaded drugs can be delivered into neutrophils by endocytosis. There are at least three forms of endocytosis that could allow internalization of NPs by neutrophils: phagocytosis, clathrin-mediated endocytosis (CME), and macropinocytosis.

6.1. Phagocytic pathway

The phagocytosis process occurs primarily in professional phagocyte cells, and is the major mechanism of NP uptake in leukocytes. Most of our understanding of the molecular mechanisms of phagocytosis comes from studies in macrophages, whereas much less is known about neutrophils, because of their resistance to genetic manipulation. Neutrophils have the ability to take up both opsonized and non-opsonized NPs. Phagocytosis of NPs is usually initiated by opsonization. Opsonization involves the binding of an opsonin, such as an immunoglobulin or antibody, complement proteins, or other blood proteins (e.g., laminin and fibronectin) to the outer surface of the NPs. Ligand-receptor interactions bring opsonized NPs into contact with and bind to phagocytic receptors. Fc receptors, complement receptors (CR1, CR3, and CR4), mannose/fructose receptors, and scavenger receptors (SR-AI /II, SR-BI, and CD36) are the major types of opsonin receptors expressed in neutrophils [137]. Recent research has shown that the Fc receptors FcγRI (CD64) and FcγRIIA (CD32) are involved in the activation of Arp2/3-dependent actin polymerization via several intermediate steps, eventually leading to phagosome formation [138]. Chu et al. found that NPs containing denatured albumin were absorbed by activated neutrophils through FcRIII, suggesting the possible involvement of phagocytosis [139]. Particle properties such as size, conformation, surface chemistry, biomechanical properties, biological conditioning (e.g., endotoxin or protein exposure) all have an impact on phagocytosis. Larger particles are often more efficiently taken up by phagocytes. For instance, particles with dimensions in the micrometer range (2–3 μm) are more easily phagocytosed by macrophages than smaller particles. Because NP diameters range from 10 to 200 nm, it is unclear whether neutrophils can phagocytose such tiny particles. It has been hypothesized that NPs form biological clumps on the outer cell membrane, which allows them to be phagocytosed [138,140].

6.2. Clathrin-mediated endocytosis (CME)

Clathrin-mediated endocytosis (CME) is a type of receptor-mediated endocytosis, because receptor binding is required for internalization. It is considered one of the major pathways for absorption of NPs and occurs in specialized plasma membrane regions recruited by clathrin. It is also involved in many other processes, including the uptake of nutrients and plasma membrane components such as cholesterol via low-density lipoproteins (LDL), and iron via the carrier transferrin. Endocytosis of nonspecific payloads can also occur through nonspecific adsorptive uptake, also referred to as receptor-independent CME, e.g., through hydrophobic and/or electrostatic interactions [141,142]. This mechanism is ideal for uptake of NPs 150–200 nm in size, but those from 250 nm to 3 μm in size are taken up via macropinocytosis or phagocytosis. However, particles that enter the cell via CME generally end up being degraded in lysosomes, and thus may not be ideal for NPs containing lysosomal enzyme-degradable materials. Several studies have investigated the absorption of different types of NPs by CME. For example, CME appears to be the major mechanism for cell entry of a few NP types, including D,L-polylactide (PLA) NPs, poly(ethylene glycol-co-lactide) (PEG-co-PLA) NPs, poly(lactic-co-glycolic acid) (PLGA) NPs, silica-based NPs, and chitosan NPs [143]. In addition, the surface charge has been observed to have a strong influence on the absorption mechanism and intracellular fate[144]. Positively charged particles are more likely to be taken up by the CME pathway than negatively charged NPs [141].

6.3. Macropinocytosis

Macropinocytosis is a type of nonspecific endocytosis in which cells take up large amounts of extracellular fluid, nutrients, or soluble compounds. It involves membranes in the form of endocytic vesicles 0.5–10 μm in diameter called macropinosomes, independent of the presence of any specific receptors. Macropinocytosis is also a mechanism for the internalization of apoptotic and necrotic cells, bacteria, and viruses [142]. Macropinocytosis allows cells to take up large particles (> 500 nm), which would not be possible with other endocytic mechanisms such as clathrin or caveolae-mediated endocytosis. Internalization of NPs by macropinocytosis occurs through close contact with the plasma membrane, generally independent of NP properties such as size or shape. Cells can thus take up NPs of different sizes simultaneously, which usually occurs in conjunction with some other mechanism[145].

7. Site-specific controlled drug release

Controlled drug delivery is a technique for increasing the therapeutic potential of chemotherapeutic agents and improving their safety and efficacy by reducing degradation, cargo leakage, and preventing removal by host cells. The concept of controlled drug delivery is to deliver drugs precisely to the target site (the tumor microenvironment or the intracellular space between cancer cells) while minimizing absorption by other host cells [146]. The development of new nanomaterials from the thriving field of nanoscience has enabled the construction of smart nanostructures that can deliver the stimulus-responsive controlled release of loaded pharmaceuticals [147]. Smart NPs can respond to a variety of different stimuli, including physical, chemical, or biological stimuli. Physical stimuli include temperature, light, magnetic field, electrical, mechanical, and ultrasound responsive NPs [148–150]. Chemical stimuli include pH and redox-responsive NPs [151], while biological stimuli include biomolecules (glucose, ATP, DNA, and ROS) and enzymes (hydrolases, proteases, trypsin) [152,153]. NPs responding to external stimuli have several advantages for drug delivery: (1) external stimuli can be precisely controlled in terms of location, amount, and timing; (2) external stimuli can be enhanced or excluded according to treatment needs; and (3) external stimuli can be applied multiple times or for longer periods to enhance response to therapy [154]. Among external stimuli, ultrasound offers a significant advantage in GBM therapy due to its deep penetration, safety, and cost-effectiveness [155]. Li et al. developed neutrophils loaded with NPs that responded to ultrasound stimulation and released drugs in a regulated manner in glioma mice models. This resulted in more effective chemo/immunotherapy against GBM and longer mouse survival without any systemic side effects [156]. The NP formulation contained a ZnGa2O4:Cr3 + (ZGO) core for sustained luminescence emission, which enabled sensitive carrier detection in the bloodstream, and a hollow sonosensitive TiO2 shell, which was used to generate reactive oxygen species (ROS) under ultrasound stimulation to control drug release [156–158]. The anti-PD-1 antibody was used as an immune checkpoint inhibitor within the core of ZGO@TiO2 in this study [159–161]. Paclitaxel (PTX)-loaded

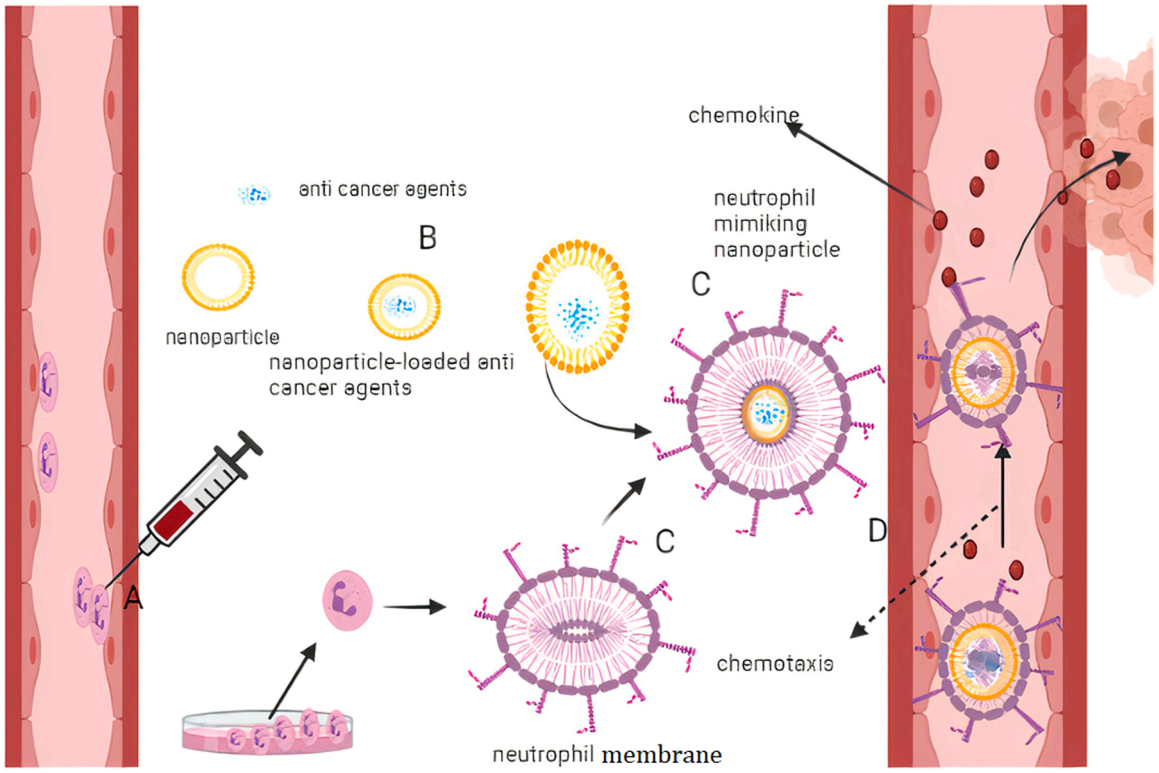

Fig. 1. Targeting of tumor cells with activated neutrophil membrane-coated NPs. Neutrophil-mediated drug delivery systems have already been identified as potential vehicles capable of transporting NPs or therapeutics to the sites of inflammation or tumors. The neutrophil membrane coating strategy may provide therapeutic NPs with neutrophil-like characteristics, and increase cancer-specific drug accumulation. Administration of NM-NPs after coating with activated neutrophil membranes, causes them to respond to higher concentrations of chemokines, a process known as chemotaxis, and accumulate in the tumor site.

liposomes were also attached to the outer surface of NPs to deliver chemotherapy to GBM tumors via the ZGO@TiO2 @ ALP complex. Neutrophils then absorbed this complex for drug delivery. Ultrasound irradiation of the GBM tumor induced the production of ROS, which disrupted the liposomal membrane, resulting in the release of PTX and the antibody PD − 1 at the GBM site to kill the cancer cells. With or without loading ZGO@TiO2 @ ALP, the procedure also caused local inflammation, which attracted more circulating neutrophils[35,156, 161].

8. Limitations and promises of neutrophil-mediated drug delivery

Numerous factors suggest that neutrophils could act as potential carriers for nanomedicine and drug delivery. These include: (1) neutrophils are the first cells to respond to signals, such as cytokines and inflammatory factors, and they are the first cells to be recruited to the site of tumors and inflammation; (2) neutrophils compared to other WBCs (such as monocytes/macrophages and lymphocytes) constitute the most common cell type during acute inflammation, which could greatly improve the efficacy of drug delivery; (3) compared with other leukocytes, neutrophils have better biocompatibility and are suitable for the delivery of a variety of cargos without triggering immune responses [162,163].

However, the use of neutrophils in clinical applications as a cell-based drug delivery approach is faced with several challenges including: (1) the extraction and purification of neutrophils from patient blood samples requires an invasive procedure that may not be possible for every patient requiring therapy; (2) the in vivo activity of many drugs and therapeutic agents inside neutrophils is still unknown [164, 165]; (3) neutrophils show limited recruitment to non-operated tumor tissue, indicating that the therapeutic effectiveness of neutrophil-mediated drug delivery is dependent on postoperative inflammation [164,166]. Furthermore, administration of large numbers of additional neutrophils raises the possibility of causing chronic inflammation. There is evidence that neutrophils play an important role in several chronic diseases, such as atherosclerosis, diabetes mellitus, nonalcoholic fatty liver disease, and autoimmune diseases. However, the risk of these chronic inflammation-related diseases arising from the injection of therapeutic neutrophils is uncertain [167]. Recently, some solutions have been proposed to overcome these challenges. It has been demonstrated that neutrophils and other cell types can be produced from induced pluripotent stem cells (iPSCs) derived from patient skin fibroblasts to avoid invasive procedures associated with neutrophil harvest. However, there are still many unresolved issues and further research is needed [168].

9. Conclusions

In this review, we have discussed how human neutrophils interact with and internalize different types of NPs such as albumin NPs, PLGA, liposomes, and magnetic mesoporous silica NPs. This is central to the strategy of using neutrophils as carriers for targeted drug delivery. We have highlighted several factors that influence the uptake of NPs by neutrophils, such as particle size and shape, concentration, type of material (liposomes, gold, albumin), surface chemistry, and the presence/absence of serum proteins. Neutrophils could be preloaded with NPs prior to injection in vitro, or the NPs could be designed to be specifically taken up by neutrophils in the bloodstream and then accumulate in brain tumors. We also reviewed the current status of neutrophil membrane-coated NPs for drug delivery, and demonstrated how innovative neutrophil-based technologies can significantly prevent tumor recurrence in GBM and lead to the complete recovery of mice. Further research is needed to reveal the full potential of neutrophil-based drug

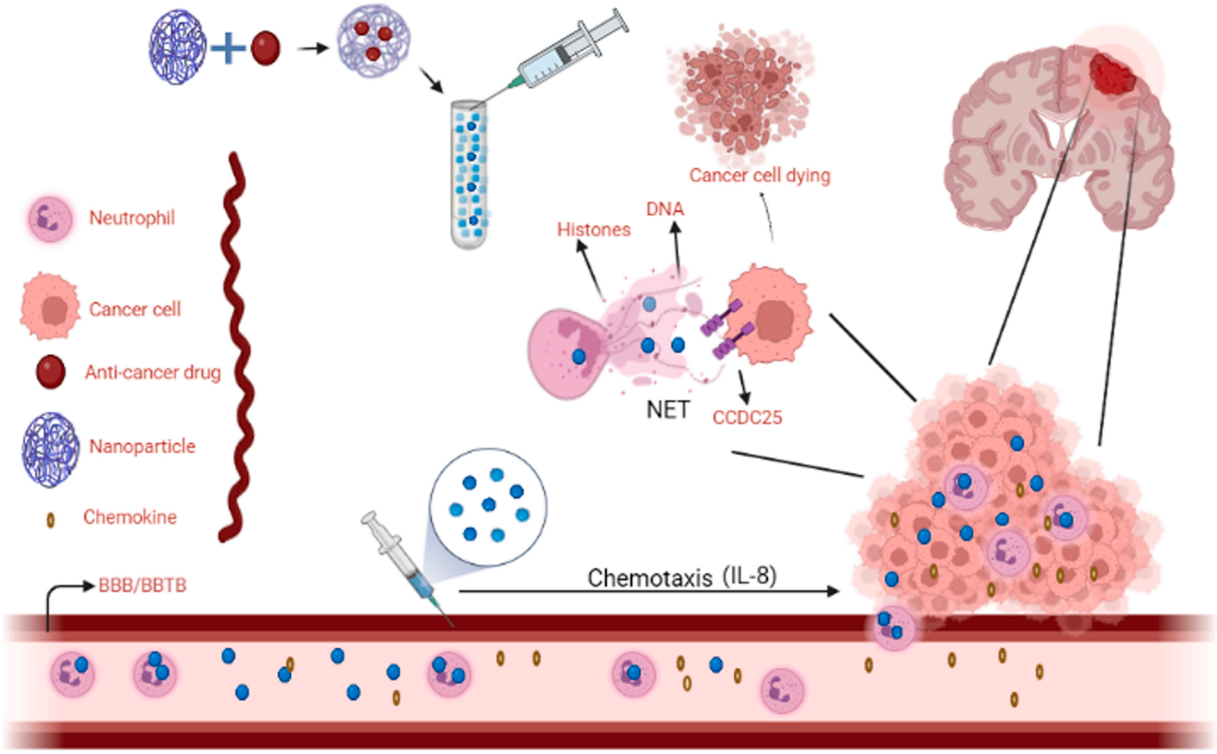

Fig. 2. Schematic illustration of neutrophil-mediated targeted delivery of NPs for GBM tumor therapy. The use of neutrophils for drug delivery has gained popularity in recent years. NPs made from FDA-approved polymers and loaded with anticancer drugs are delivered into the bloodstream. Intravenously administered NPs are taken up by activated neutrophils, which then migrate across the blood-brain barrier into the inflamed brain and penetrate the residual tumor. Inflammatory cytokines (e.g., IL −8) released from the residual tumor create a chemotactic gradient that drives neutrophil migration to the tumor site. The concentrated cytokines excessively activate the neutrophils. Activated neutrophils exert anticancer activity by forming neutrophil extracellular traps (NETs) that lead to the simultaneous release of NPs and delivery of chemotherapeutic agents to the residual tumor cells.

delivery in GBM therapy. Fig. 1 and 2.

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

CRediT authorship contribution statement

Hamed Mirzaei involved in conception, design, and drafting of the manuscript. Hamed Hosseinalizadeh, Mehrdad Mahmoodpour, Michael R Hamblin contributed in data collection and manuscript drafting. All authors approved the final version for submission.

Conflict of interest statement

MRH declares the following potential conflicts of interest. Scientific Advisory Boards: Transdermal Cap Inc, Cleveland, OH; Hologenix Inc. Santa Monica, CA; Vielight, Toronto, Canada; JOOVV Inc, Minneapolis-St. Paul MN; Sunlighten, Kansas City, MO; Consulting; USHIO Corp, Japan; Sanofi-Aventis Deutschland GmbH, Frankfurt am Main, Germany. Stockholding: Niraxx Light Therapeutics, Inc, Irvine CA; Jelika-Lite Corp, New York NY. The other authors have no relevant financial or non-financial interests to disclose.

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