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Cell-Mediated Drugs Delivery

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Abstract

INTRODUCTION—Drug targeting to sites of tissue injury, tumor or infection with limited toxicity is the goal for successful pharmaceutics. Immunocytes (including mononuclear phagocytes (dendritic cells, monocytes and macrophages), neutrophils, and lymphocytes) are highly mobile; they can migrate across impermeable barriers and release their drug cargo at sites of infection or tissue injury. Thus immune cells can be exploited as trojan horses for drug delivery.

AREAS COVERED IN THIS REVIEW—This paper reviews how immunocytes laden with drugs can cross the blood brain or blood tumor barriers, to facilitate treatments for infectious diseases, injury, cancer, or inflammatory diseases. The promises and perils of cell-mediated drug delivery are reviewed, with examples of how immunocytes can be harnessed to improve therapeutic end points.

EXPERT OPINION—Using cells as delivery vehicles enables targeted drug transport, and prolonged circulation times, along with reductions in cell and tissue toxicities. Such systems for drug carriage and targeted release represent a novel disease combating strategy being applied to a spectrum of human disorders. The design of nanocarriers for cell-mediated drug delivery may

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Article Highlights

- Using cell-mediated drug delivery systems offers several advantages including: a) targeted drug transport to disease sites; b) prolonged drug half-lives; c) time-controlled release of loaded drugs; and d) diminished drug immunogenicity and cytoxicity profiles.
- The goals need to be achieved for successful cell-mediated drug delivery include: a) high drug loading into cell-carriers; b) efficient preservation of entrapped therapeutic agents against disintegration and clearance in the host cells; c) drug triggered release at the site of action; d) efficient homing of cell-carriers to a disease site; e) safety of cell-based drug formulations for the whole organism.
- Two different approaches are utilized in cell-mediated drug delivery: genetically modified cell-carriers producing therapeutically active molecules; and drug loaded cell-carriers used as “Trojan horses” to deliver the drug to the disease side.
- Living cells for drug carriage and release represent a novel disease combating strategy that can be applied to a spectrum of human infectious, cancerous, and degenerative disorders.

differ from those used for conventional drug delivery systems; nevertheless, engaging different defense mechanisms into drug delivery may open new perspectives for the active delivery of drugs.

Keywords

cell-carriers; *drug delivery*; *immunocytes*; *nanoparticles*; *targeted drug transport*

Introduction

The development of targeted drug delivery is amongst the most important goals of pharmaceutical research. Its realization can lead to improved therapeutic efficacy, reductions in drug dosing intervals, and decreased toxicities. However, this is not a simple task as drug homing to pathologically relevant disease sites has only recently been investigated. Obstacles are substantial and include sustained time-based plasma concentrations and local blood flow.

1. Promise and Perils of Cell-Mediated Drug Delivery

Using immunocytes, mononuclear phagocytes (MP; monocytes, macrophages, and dendritic cells) lymphocytes, and neutrophils and stem cells as drug carrier systems offers several advantages over common drug administration regimens. These include targeted drug transport to disease sites; prolonged drug half-lives; time-controlled drug release; and diminished drug immunogenicity and cytoxicity profiles. Immunocytes and stem cells exhibit an intrinsic homing property enabling them to migrate to sites of injury, inflammation, and tumor. In addition, they can act as Trojan horses carrying concealed drug cargoes while migrating across impermeable barriers (for example, the blood brain or blood tumor barriers) to sites of disease.

Despite such advantages, there is as yet limited success for several reasons. *First*, drug loading in cell carriers is low. *Second*, there are limitations due to the ability of immunocytes and stem cells to efficiently disintegrate and clear entrapped therapeutic agents. *Third*, the loaded drug should not be prematurely released, but unloaded, in continuous action, upon the cell's arrival to the site of action or disease. *Fourth*, the cell carriers should migrate to the disease site in substantial quantities. This should not be compromised during drug loading. *Finally*, all used formulations must be safe for both the cell carrier and the organism.

Many of these limitations can be addressed by incorporation of drugs into protective polymeric nanocarriers (liposomes and lipid nanoparticles [1-4], micelles [5-8], nanogels [9, 10], nanospheres and nanocapsules [11, 12], solid nanoparticles and nanosuspensions [13-16], block ionomer complexes [17, 18] or nanofibers and nanotubes [19, 20]) that preserve drugs inside subcellular organelles (Figure 1). The ideal drug carrier for cell-mediated delivery should have optimal size, shape and surface characteristics; multivalent attachment; controlled drug release; and biocompatibility. All these characteristics are essential for translating cell-mediated drug delivery systems for human use.

Drug loading into cell-carriers—Nanocarriers commonly have a core-shell structure. Their central part (such as aqueous pool of nanospheres, hydrophobic core of micelles, or polyelectrolyte core of nanogels) permits drug entrapment. It is surrounded with a polymer shell (or lipids in liposomes and lipid nanoparticles), which defines the nanocarrier dispersion stability, circulation time and cell interactions [1-8, 10, 11, 13, 14, 17, 18]. Both the core and shell are crucial for successful cell-mediated delivery. For example, the surface coating affects the ability of particles to internalize into the cells and therefore, affects the

loading of cell-carriers. In general, charged nanocarriers are rapidly taken up by MP and other immunocytes or stem cells compared to neutral nanoparticles [1, 21-25]. The recognition occurs by receptors located on the cell plasma membranes, in particular, mannose, complement, and Fc receptors (MR, CR and FcR) [26-28]. In general, MR recognize mannans, as well as integrins (for example CD11b/CD18), CR interact with particles after nonspecific complement opsonization, and FcR recognize particles after specific antibody opsonization [28]. Furthermore, positively charged nanoparticles accumulate in MP to a greater extent than negatively charged particles. Thus, positively charged nanoparticles prepared by high pressure homogenization with antiretroviral drugs, indinavir (IDV), ritonavir (RTV), and efavirenz (EFV) accumulate in MP at about two-fold greater than the same size negatively charged carriers [14]. Similar effect was reported for nanoparticles ("nanozymes") comprising of redox enzyme, catalase, and variant synthetic polyelectrolyte block copolymers [29]. Loading capacity of nanozymes comprising of positively-charged mono-polymers (polyethyleneimine-(PEI) and poly-L-lysine- (PL) based) is greater than the nanozyme prepared with negatively-charged block poly-L-glutamic acid (PGLU)-based copolymer, This likely occurs due to the greater absorption and internalization of positively-charged nanoparticles to negatively-charged outside plasma membrane of cell-carriers.

An electrostatically neutral and hydrophilic poly(ethylene glycol) (PEG) is perhaps the most common shell-forming polymer currently used in injectable nanocarriers [30-32]. Particularly, commercial PEGylated liposome-encapsulated Dox, Doxil, is approved for use in the treatment of recurrent ovarian cancer, AIDS-related Kaposi's sarcoma [33] and metastatic breast cancer [34]. However, PEG is by far not the only polymer that should be considered in the context of the cell-mediated delivery, as it can limit cellular uptake as was demonstrated for microspheres bearing high-density surface PEG chains that were resistant to phagocytosis [35]. Similar results were obtained with nanoparticles comprised of catalase and positively charged polymers based on PEI [29]. Thus, loading capacity of nanozymes without PEG corona in MP was significantly greater than those with PEG. To this end, modification of nanocarrier shell with specific vector moieties can provide for targeted cell delivery of nanocarriers [1, 26, 36-38]. In particular, modification of PEG shell of a poly(amidoamine) nanoparticles (PAMAM) with streptavidin facilitates targeting to biotinylated T-cell markers such as anti-CD3 or peptide/ major histocompatibility (MHC) complexes [39] (Figure 2).

Size and shape of nanocarriers are also of importance for cell uptake, although the phagosome may have different sizes depending of the size of the particles, which can range from as little as few hundred nanometers [35, 40, 41] to several of microns [42]. For example, murine bone marrow-derived macrophages accumulate IgG-opsonized latex beads greater than 20 μm in diameter [43]. Furthermore, particles with the size about 1μm were accumulated at greater extent (2.56 fold) than smaller drug carriers at 500 μm [14].

A recent study by Champion et al. reported striking effects of shape of particles on phagocytosis in alveolar rat macrophages [44]. Polystyrene-based particles of more than twenty shapes including spheres, rectangles, rods, worms, oblate ellipses, elliptical disks and UFO-like particles were manufactured. The local particle shape at the point where it was attached to the cell played the crucial role in phagocytosis. For example, a macrophage attached to a sharper side of the ellipse would internalize the particle in a few minutes. In contrast, a macrophage attached to a dull side would not internalize the same ellipse for hours. Although, particle size played a reduced role in the initiation of the phagocytosis it could of course affect its completion especially when the particle volume exceeded that of a cell.

Drug preservation inside cells—The stability of the drug loaded into cell carriers among other factors can depend on the intracellular trafficking of the nanocarriers. In

general, drug-loaded nanocarriers need to avoid lysosomes to reduce drug disintegration inside cells [14]. In this regard, it is noteworthy that cationic and anionic nanoparticles show divergent fates inside MP [38]. For example, phagocytosis of cationic polyamine-coated nanoparticles leads to the diminished phagosomal acidification when compared to anionic protein-coated particles. Such cationic nanoparticles protect the incorporated drug against lisosomal degradation [29, 38]. Furthermore, loading of "nanozymes" containing positively-charged block copolymers (PEI-PEG and PL-PEG) protected the enzyme in macrophages. Increasing the amount of the block copolymer in the nanozyme formulation improved the stability of the enzyme. In contrast, catalase loaded in a polyion complex with a negatively charged block copolymer (PGLU-PEG) was degraded in macrophages to an even greater extent than catalase loaded alone. Protection of the enzymatic activity inside carrier cells may be, in part, due to a "proton sponge" effect of block copolymers [45], when an excess of amino groups on the surface of the nanoparticles buffers acidification of the cell's endocytic compartments. This serves to inhibit protease activity and decrease drug degradation [38]. In other words, a positively charged block-copolymer prevents phagosome-lysosomal clearance functions and as a result, enzyme degradation.

In extreme case, intracellular drug degradation can be avoided by attaching the drug to the surface of cell-carriers. This "back pack" approach would still provide targeted transport and increased blood circulation along with the preserved drug activity. Thus, attaching avidin-coated nanoparticles to the biotinylated plasma membrane can be achieved through the avidin-biotin complex [46]. Another possibility is red blood cells (RBCs) with attached drugs on their surface [47]. Specifically, glycoprotein A covalently conjugated to the surface of the RBCs may provide extended half-life, controlled volume of distribution, and multivalent therapeutic interactions. However, general limitations of the "back pack" approach may include decreased loading of cell carriers, impeded drug release at the disease site, as well as increased immunogenicity and toxicity.

Drug release from cell-carriers—Mechanism of nanoparticle unloading at the site of action remains an active area of investigation. Controlled release of drugs from the cell-carriers modulates the rate of drug appearance, dose and duration of exposure at the diseased sites. To this end, utilizing cellular responses to various conditions could provide desired triggered release. Obviously, targeting of cell-carriers and their prolonged residence at the disease site should provide opportunities for drug unloading. In addition, MP, in particular, are known to produce and store various compounds in intracellular vesicles, and liberate them *via* exocytosis at the site of the disease. A similar mechanism can trigger release of drug-loaded nanocarriers, when macrophages serve as drug delivery vehicles [48]. Furthermore, the drug release can be also triggered by the increase of intracellular concentration of Ca^{2+} [49]. Finally, mild hypothermia was shown to facilitate controlled release of drug-loaded liposomes from macrophages in anti-cancer therapy [50].

Overall the structure and composition of protective nanocontainers play a crucial role in the effectiveness of formulations for cell-based drug delivery systems. For example, our recent studies indicate that structure of block copolymer used for catalase nanoformulation, nanozyme, affect its cytotoxicity, loading and release capacities, as well as preservation of catalase enzymatic activity inside cell-carriers [29]. Thus, nanozymes containing a negatively-charged block copolymer (PGLU-PEG/catalase) demonstrated low toxicity, high loading capacity and effective release from macrophages. However, the polymer provided limited protection of the enzyme against cell-associated protease degradation. In contrast, nanozymes based on positively charged block copolymers, especially the PLs, showed increased cytotoxicity and low loading and release rates, but were protective of the catalase. Increasing the amount of positively-charged block-copolymer in nanozyme leaded to protection of catalase enzymatic activity but substantially reduced loadings and release.

Importantly, nanozymes with PEG corona show water stability, limited cytoxicity and efficient catalase protection. Nonetheless, these formulations also demonstrated decreased loading capacity and release rates. In addition, nanozymes based on mono-polymer (without PEG corona) have higher loading and release levels, but did not protect catalase inside the cell-carriers. Taken together, the most optimal nanozyme formulation was the one based on positively charged block copolymers (PEI-PEG/catalase and PL-PEG/catalase) that demonstrate the most efficient protection of catalase enzymatic activity along with relatively high loading and release rates with limited cytotoxicity (Figure 3).

Homing of drug-loaded cell-carriers at disease sites—The numbers of cell-carriers that can reach the disease site is especially crucial in the case of CNS disorders when drug-loaded cells need to penetrate the blood brain barrier (BBB) to mediate therapeutic effect. Many neurological diseases, such as Alzheimer's and Parkinson's diseases (AD and PD), Prion disease, meningitis, encephalitis and HIV-associated neurocognitive disorders (HAND), have in common an inflammatory component [51]. The process of inflammation is characterized by extensive MP recruitment. Notably, MP migrate toward the inflammation site *via* the processes known as diapedesis and chemataxis [52]. Such cells efficiently cross the BBB due to their margination and extravasation properties causing barrier breakdown as a consequence of brain inflammation[53-57]. Many reports in the literature indicate that blood borne monocytes traffic primarily between adjacent endothelial cells, *i.e.* paracellularly through the junctional complexes[58, 59]. Therefore, these cells can be loaded with a required drug and administered intravenously to reach the brain. For example in an experimental model of PD, considerable levels of catalase loaded into BMM were reported in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-intoxicated brains[48]. Approximately 2.1% of the injected catalase dose was delivered to the brain during MPTP-associated inflammation. Furthermore, several studies confirmed the migration of inflammatory-response cells, in particular, bone-marrow-derived mesenchymal stromal cells toward injury sites such as infarcted myocardium[60, 61], spinal cord injury[62], and cerebral ischemia[63].

Neural stem cells (NSCs) were also suggested as drug delivery vehicles for gene therapy in the CNS [64-66]. Indeed, these cells are highly migratory and migrate to areas of brain pathology including ischemic and neoplastic brain lesions that are commonly present in AD, PD, brain cancer, stroke, and multiple sclerosis. How the mobility of stem cells are directed is not understood, although NSCs express a wide variety of receptors that should enable them to respond to many chemotactic signals present in brain pathologies (Figure 4) [66]. In particular, activated microglia induced NSCs migration to the brain. Delivery of various neurotropic factors is in the focus of cell-mediated strategies for neurological diseases treatment.

In regards to cell-mediated drug delivery of antineoplastic agents, MP are known to accumulate in large numbers in vascular, hypoxic sites in cancer tumors, for example, in breast and prostate carcinomas [67, 68]. Hypoxia is widespread in malignant human tumors due to their poorly organized vasculature. The cytokines released by tumor cells in response to hypoxia and other physiological stresses usually attract macrophages and monocytes, which should facilitate anticancer drug delivery using these cells as vehicles, avoiding indiscriminate drug distribution and decreasing severe toxicity.

Finally, targeting to disease sites can be improved by drug-incorporated magnetic nanoparticles loaded into the cell-carriers and followed by application of local magnetic fields [37]. Thus, albino rats with brain inflammation (induced by intrastrial microinjection of human recombinant IL-1β) received intravenous injections of RGD-coated magnetic liposomes and non-magnetic liposomes as a control. RGD peptide (*i.e.* small peptide domane Arg-Gly-Asp) was used for selective binding by monocytes and neutrophils that

express integrin receptors on their surface. Magnetic liposomes demonstrated about a ten-fold increase in brain levels compared with non-magnetic controlled carriers when local magnetic field was applied. In addition, magnetic neutrophils prepared *in vitro* target lungs under magnetic guidance following intravenous injection [69]. Moreover, drug loaded magnetic liposomes can be targeted for selective and preferential presentation to blood monocytes/neutrophils that result in both drug and magnetic incorporation into these cells and can be guided to target tissue sites.

Safety of cell-mediated drug delivery systems—An obvious concern for inflammatory-response cell based drug delivery relates to possible cytotoxic effects of cell-carriers. MP attracted to the site of pathology by cytokines release reactive oxygen species (ROS) that cause cell damage. Moreover, a number of therapeutic strategies for CNS neurodegenerative disorders are based on the prevention of monocyte-macrophage infiltration [70]. Therefore, precluding cytotoxicities for cell-based drug formulations is a requirement for formulation developments. Furthermore, genetically-modified and immortalized cell-carriers show atypical characteristics, such as higher degree of multipotency, which may increase the probability of tumor formation. Nevertheless, studies so far report no cytotoxic effects after macrophage-mediated drug delivery in the brain of healthy C57BL/6 mice adoptively transferred with macrophages carrying nanoformulated catalase [71]. In addition, propagation and expansion of immortalized and genetically-modified cell-carriers should allow improve quality controls [66].

Cell-mediated drug delivery systems in clinical settings—Finally, developing such methods in a clinical setting may be successful when cell-carriers are harvested from peripheral blood by apheresis, then loaded with particles and re-infused into the patient. An alternative approach may be harvesting stem cells from bone marrow, propagation them in culture to obtain monocytes, and then loading the cells with a drug and adoptively transferring them. This procedure will allow for expansion of the cell population, although this would require a more invasive procedure. To this end, immortalization of cell-carriers can allow propagation of cells with definable properties so that clonal populations with particular traits can be established. Furthermore, drug loading into cell-carriers can be achieved directly in the patient's peripheral blood, when nanoformulated drugs would be injected and selectively taken up by circulating cell carriers. Targeting of such nanocarriers could be achieved by coating the nanocarrier surface with the receptor-specific moieties (e.g., folate, gelatin, fibronectin, A-protein, mannose, or RGD peptide), which is recognized by specific MP surface receptors. Overall, chronic diseases are more suitable for this type of drug delivery than pathologies that require acute intervention. Based on such considerations, we now present some successful investigations using living cells as drug delivery vehicles for therapeutics (Table 1).

2. Neurodegenerative disorders and cell-based carriage of therapeutic nanoparticles

AD and PD—The progressive impairment of short-term memory and emotional disturbances that typify AD results from synaptic dysfunction and neuronal death in the hippocampus and linked regions in the cerebral cortex and limbic system. Because of the increasing numbers of affected people, there is an urgent need to develop strategies able to interfere with disease progression and protect neurons. In this respect, delivery of neurotrophic factors (NTFs) including nerve growth factor (NGF) [65], brain-derived neurotropic factor (BDNF) [64], and choline acetyltransferase (ChAT) [72] is of urgent need. To overcome limited drug transport across the BBB, direct brain implantation of cells engineered to produce NTFs was developed [73]. In particular, ciliary neurotrophic factor delivered in cells implanted in the brain prevents Aβ oligomer-induced neuronal damage and neurobehavioral impairments in mouse models of AD. In another study NSCs

overexpressing ChAT cDNA targeted acetylcholine deficits [74]. The ability cell-carriers, placed by cortical transplants, to improve cognition was tested after induced cholinergic lesions in rats. Significant improvements were recorded in Water maze acquisition as well as in the retention and spatial probe trials [74]. A robust enhancement of hippocampal synaptic density, mediated by BDNF and delivered by NSCs was seen [75]. NSCs ameliorated complex behavioral deficits associated with AD pathology by BDNF. In these experiments the intracranial transplantation of the cell-carriers were utilized.

Due to specific nigrostriatal degeneration, PD was one of the first targets for cell therapy transplants. Fetal dopamine neurons were inoculated into the putamen, where the cells worked as dopamine pumps as seen during systemic administration of L-DOPA [76]. It was suggested that the transplanted cells were gene therapy vehicles for dopamine delivery rather than replacement neurons [76]. Delivery of neurotropic factors such as glial cell-line derived neurotropic factor (GDNF) or vascular endothelial growth factor (VGEF) in neural stem cell transplants were tested in PD mouse models [77-79]. GDNF was delivered in bone marrow-derived macrophages to the effected brain [80]. The cells were transduced *ex vivo* with lentivirus expressing a GDNF gene driven by a synthetic macrophage-specific promoter and then transplanted into recipient mice. Eight weeks after transplantation, the mice were injected with MPTP for seven days to induce nigrostriatal neurodegeneration. Macrophage GDNF treatments dramatically ameliorated MPTP-induced degeneration of tyrosine hydroxylase (TH)-positive neurons seen in the substantia nigra and TH(+) terminals in the striatum. This resulted in axon regeneration and reversed hypoactivity in the open field test.

Development of novel CNS drug delivery using macrophages carriers for delivery of the antioxidant enzyme, catalase, in MPTP mice was reported by our laboratories [48, 71]. In this system, nanoformulated catalase (nanozyme) was obtained by coupling the enzyme to a cationic block copolymer, PEI-PEG, leading to a polyion complex micelle. Bone marrow-derived macrophages (BMM) carried significant amounts of catalase then slowly released the active enzyme over several days [48]. The enzyme released upon stimulation of nanozyme-loaded cell-carriers decomposed microglial hydrogen peroxide produced upon nitrated alpha-synuclein (N-α-syn) or tumor necrosis factor alpha (TNF-α) induced activation *in vitro*. Subsequent studies examined relationships between the composition of catalase nanozyme, their physicochemical characteristics (morphology, size, and ξ-potential), and cell loadings, release, and enzymatic activities for macrophage carriage [29].

Significant amount of catalase was detected in brains of mice after transfer of BMM loaded with nanoformulated catalase following MPTP intoxication. It was demonstrated that such nanozyme-loaded BMM injected into MPTP-intoxicated mice reduce neuroinflammation and attenuate nigrostriatal degeneration [71]. In particular, MPTP intoxication decreased the number of TH-positive nigral dopaminergic neurons (32 % survival) compared to saline-treated controls (Figure 5). In contrast, the number of surviving dopaminergic neurons in MPTP-intoxicated mice treated with nanozyme-loaded BMM was greater than the total number of neurons in MPTP-treated mice (62.4 % survival). Furthermore, treatment with nanozyme alone (without cell carriers) also produced some neuroprotection effect, although with fewer neurons (41.3 % survival) compared to the mice treated with cell/nanozyme formulation. Finally, treatment with empty monocytes did not preserve neurons in MPTP-intoxicated animals (31.1 % survival). This signified a neuroprotective effect of nanozyme-loaded monocytes in MPTP-induced neurodegeneration.

The possible means by which BMM-mediated therapeutic effects of the nanozymes were suggested (Figure 6) : 1) nanozyme-loaded BMM cross the BBB and release catalase at the site of inflammation (particularly, in the substantia nigra pars compacta, *SNpc*); 2) a “depot”

was established such that nanozyme is slowly released from BMM to the blood stream and bypasses the BBB independently of cell-carriers; and 3) catalase nanozyme released from BMM in the liver and spleen suppresses peripheral leukocyte activation that results in significant protection of SNpc neurons against MPTP-induced neurodegeneration [71]. Overall, as few therapeutic modalities exist which affect progression of PD and aimed at neuroregenerative therapies, the cell-mediated delivery of catalase and GDNF may represent efficacious strategies that attenuate neuroinflammation and provide neuroprotection for disease.

HIV-1 infections and neuroAIDS—NeuroAIDS is a clinical disorder that encompasses neurologic disorders seen primarily as a consequence of damage to the central nervous system by HIV. The clinical syndromes include cognitive, motor and behavioral disorders of varying severity. Such syndromes affect 30 to 40% of adults and children with AIDS and, despite the advent of potent combination antiretroviral therapy (cART), incidence rates remain constant although disease severity is less. MP carriage has been utilized as a delivery system for antiretroviral therapy of HIV in mice [14, 57, 81-83]. To protect the drug against degradation, as well as the cell-carriers against the drug, it was incorporated into nanosized drug crystals (termed nano antiretroviral therapy or nanoART, Figure 7). NanoART were made by high-pressure homogenization of crystalline drug with various poloxamer and/or phospholipid surfactants, or by wet-milling homogenization, which produced nanoformulations with high loading capacities (over 80%) and relatively small particle sizes (200 to 350 nm). Scanning electron microscopy analysis revealed particles of various sizes and morphologies, which were either polygonal, rod, cuboidal, or spherical in shape with smooth-surfaces.

Simultaneous uptake of nanoformulated cART into the cell-carriers was shown [83]. It was also demonstrated that size, charge, coating, and shape of the nanoparticles are crucial for efficient MP uptake, drug release, and cell migration [14]. Coating of nanoART greatly affected drug accumulation in cell-carriers. Positively-charged nanoART are taken up better than negatively charged ones. To determine whether any of the wet-milled nanoformulations could induce long-term antiretroviral effects, macrophages were pretreated with individual nanoART preparations and then challenged with HIV-1_{ADA} up to 15 days after drug treatment. Nanoparticles loaded with atazanavir, ritonavir and indinavir were released from macrophages and demonstrated >85, 80 and 40% inhibition, respectively, of progeny virion production and HIV-1p24 antigen through challenge day 15. All formulations of EFV showed almost complete suppression of viral infection [83]. These results supported the continued development of macrophage-mediated nanoART carriage for treating HIV-1 disease. In addition, delivery of antisense oligodeoxynucleotides, ribozymes, incorporated into liposomes to HIV-1-infected cells was reported in human monocyte-derived macrophages [84]. Thus, in addition to nanoART a ribozyme complementary to HIV-1 5'-long terminal repeat delivered in pH-sensitive liposomes inhibited virus production by 90%, while the free ribozyme caused limited viral inhibitions.

3. Cancer

Cell-mediated delivery is also a potential therapeutic and diagnostic strategy for cancer [46, 50, 85-93]. The advantage herein is that cytotoxic activity of the cell-carriers can be used in tandem with site-specific delivery of antineoplastic agents. In addition, antineoplastic agents are safely packaged into cell-carriers to reduce secondary cytotoxicities. However, adverse effects of the cytotoxic drugs on the cell-carriers themselves need be considered. For example, when nanoparticles coated with cytotoxic antibiotic, doxorubicin, were loaded into T-cells, the release of drug inside the cell-carrier caused cell damage [94]. As a result over 60% T cells loaded with the nanoparticles were dead over 15 hours after the loading.

To overcome these limitations, mesenchymal stem cells (MSCs) were genetically engineered to produce antitumor proteins [86, 90-93, 95]. MSCs possess a set of several unique properties, which make them ideally suited for both cellular therapies in regenerative medicine and as vehicles for gene and drug delivery to treat cancer. These properties include: 1) relative ease of isolation; 2) the ability to differentiate into a wide variety of functional cell types of both mesenchymal and non-mesenchymal origin; 3) the ability to be extensively expanded in culture without loss of differentiative capacity; 4) the hypoimmunogenicity and even ability to produce immunosuppression upon transplantation; 5) the pronounced anti-inflammatory properties; and last but not the least, 6) the ability to home to damaged tissues, tumors, and metastases following *in vivo* administration. Thus, MSCs of human origins were genetically modified to express TNF related apoptosis-inducing ligand (TRAIL) at the glioma tumor site. First, the retention of tumor tropic ability of hMSC S-TRAIL cells by *in vitro* and *in vivo* migration assays was clearly demonstrated [93]. Next, for the *in vivo* assessment of therapeutic efficacy, hMSCs were injected ipsilateral to an established intracranial glioma tumor in a mouse xenograft model. Genetically engineered hMSC S-TRAIL cells were effective in inhibiting intracranial U87 glioma tumor growth (81.6%) *in vivo* and resulted in significantly longer animal survival. Immunohistochemical studies demonstrated a significant, 8-fold greater tumor cell apoptosis in the hMSC S-TRAIL-treated group compared to controls. Overall, the study demonstrated the therapeutic efficacy of hMSC S-TRAIL cells and confirmed that hMSCs can serve as a powerful cell-based delivery vehicle for the site-specific release of therapeutic proteins. Furthermore, reduction of tumor growth by genetically-modified hMSCs expressing interferon-β was reported in models of metastatic breast cancer, melanoma [86, 90], and gliomas [95]. The hMSC were also used as carriers for conditionally replicating adenoviruses in a breast cancer metastasis model [91] and in a model of intracranial malignant glioma [92].

Attachment of nanoparticles with cytotoxic agent to the cell surface allowed cell carriers to remain intact. Thus, NeutrAvidin-coated nanoparticles were anchored on biotinylated plasma membrane of hMSC where they remained attached for up to two days [46]. The hMSC with such nanoparticulate patches retained their inherent tumoritropic properties in a tumor model with a 3D-extracellular matrix. These results provide a novel strategy to actively deliver nanostructures and therapeutics to tumors utilizing stem cells as carriers.

Besides MSCs, erythrocytes, MP, and bacterially-derived minicells were also evaluated as carriers for delivery of antineoplastic agents. In particular, erythrocytes were used as carriers for anticancer agent, 5-fluorouracil (5-FU), to treat malignant ascites [96]. Intravenous injections of the cell-carriers loaded with 5-FU resulted in significant regression of the quantity of malignant ascites and the increase of survival time in mice. Notably, MP can serve as “Trojan Horses” for nanoparticle transport into tumor regions [85, 89]. Thus, infiltration of monocytes and active delivery of Au-containing nanoparticles to the center of tumor spheroids (which contain hypoxic centers) was reported [89]. Following the delivery the tumor spheroids were destroyed by irradiation with near-infrared light, which actuated the delivered Au-containing nanoparticles. In another study, macrophages acted as a cellular vehicle for 5-FU encapsulated in oligomannose-coated liposomes (OMLs) [50]. The successful decrease of tumor growth by co-administration of OML-encased 5-FU and OML-encased magnetic nanoparticles, followed by treatment with an alternating magnetic field was reported in a mice. These OMLs were injected into the peritoneal cavity, and then gradually accumulated in the omentum and the other lymphoid tissues within 24 hours. Treatment of macrophages at 39 °C for 30 min. led to the release of 5-FU from the macrophages, suggesting that controlled release from macrophages could be achieved by mild hyperthermia. The encased magnetic nanoparticles, which are known to convert electromagnetic energy to heat in the OMLs allowed achieving *in vivo* hyperthermia. Finally, MP were utilized for delivery of therapeutic DNA constructs into tumors [85].

Bacterially-derived minicells with diameters of 400 nm were suggested for targeted delivery of different chemotherapeutics and small interfering RNA (siRNA) duplexes that cause stabilization and regression of tumor size in human cancer xenografts in mice [97, 98]. The cell-carriers were specifically targeted to tumor cell-surface proteins with biospecific antibodies (BsAb). About 80% efficiency of packing minicells with siRNA was reported. Thus, minicells carrying at least 12,000 siRNA molecules and 100 copies of short hairpin RNA (shRNA)-encoding plasmid can specifically and sequentially deliver to tumor xenografts these therapeutics and compromise drug resistance by knocking down a drug resistance protein. This cell-mediated drug delivery system enables to use several thousand-fold less drug than needed for conventional systemic administration of cancer therapies. Evaluation of uptake and intracellular kinetics of minicell-delivered siRNA indicates that after endocytosis, the minicells traverse the well-established early and late endosomal pathways, terminating into acidified organelles, the lysosomes, where mini-cells are degraded and release their cargo[98]. In addition, several methods of packaging in minicells of a range of anticancer chemotherapeutic drugs, such as doxorubicin, paclitaxel, irinotecan, 5-FLU, cisplatin, carboplatin monastrol, and vinblastine despite their disparate structure, charge, hydrophobicity, and solubility were developed [99-101].

Antibody-producing hybridoma cells were used for delivery of anti-CD137 and anti-OX40 mAb [102]. Microcapsules containing viable cells that secrete antibody were implanted by injection into the subcutaneous tissue of Balb/C mice bearing CT26 colon carcinomas. CD137 and OX40 are members of the TNF receptor family that provide costimulatory effect on T lymphocytes. The treatment resulted in complete tumor eradication in an elevated fraction of cases and strong tumor-specific cytotoxic T lymphocyte responses with either anti-CD137 or anti-OX40 producing hybridomas.

Besides cytotoxic agents, delivery of imaging agents is of importance for cancer therapy. MP-mediated delivery of quantum dots to brain tumors was explored for this purpose [103]. Thus, the surgical management of brain tumors requires the precise localization of tumor tissues within normal brain parenchyma in order to achieve accurate diagnostic biopsy and complete surgical resection. To this end, quantum dots are optical semiconductor nanocrystals that exhibit stable, bright fluorescence. It was demonstrated that the intravenous injection of quantum dots is accompanied by reticuloendothelial system and macrophage sequestration. Macrophages infiltrate brain tumors and phagocytize intravenously injected quantum dots, optically labeling the tumors.

Another class of imaging agents, *N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-gadolinium (Gd) chelates, were used for enhanced magnetic resonance imaging (MRI) of macrophage-mediated malignancies [104]. To increase the nanoparticles uptake in macrophages, copolymers were vectorized with mannosamine, which was shown to increase mannose receptor mediated uptake in human MP. It was suggested that intravenously administered GD-nanoparticles will be accumulated in macrophages and then transported to the pathological sites (e.g., solid tumor). Overall, the tumor recruitment of inflammatory-response cells may be exploited for cell-mediated delivery of antineoplastic and imaging agents.

4. Lung Injury

Drug delivery to small airways, terminal bronchioles and alveoli is complicated due to the methodological limitations in targeting the deep lung with high efficiency drug distribution to the site of pathology. To overcome these limitations, chitosan nanoparticles with entrapped curcumin (an anti-inflammatory compound) was loaded into Testis-derived Sertoli cells and then injected intravenously into mice with bronchial or alveolar injuries[105]. By 24-hours post-injection, most of the curcumin load (~90%) delivered in the injected

Sertoli cells was present and distributed throughout the lungs including the perialveloar sac area in the lower lungs. These results identify a novel and efficient method for targeted delivery of drugs for treatment of bronchitis and alveolitis. In addition, lungs are suitable for both local and systemic drug delivery, therefore this route may be used broadly for other diseases.

5. Microbial Infections

The efficiency of antifungal agents has been diminished by their severe side effects and poor pharmacokinetics. To this end, erythrocytes were suggested as delivery vehicles for antifungal agent, amphotericin B (AmB). In order to avoid toxic effects and achieve efficient drug loading, AmB was encapsulated in nanosuspension (AmB-NS) by high-pressure homogenization [106]. AmB-NS was loaded into RBC by using hypotonic hemolysis, leading to intracellular AmB amounts of 3.81 +/- 0.47 pg RBC^{-1} and an entrapment efficacy of 15-18%. Hence, RBC served as primary carriers. Then, the uptake of RBC loaded with AmB-NS by leucocytes was studied by flow cytometry. More than 98% of the phagocyte population (granulocytes and peripheral monocytes accumulated AmB-NS after four hours of incubation, and then showed a slow AmB release over ten days without any alteration in cell viability. This results in an immediate, permanent inhibition of intra- and extracellular fungal activity. AmBNS-RBC-leukocyte-mediated delivery of AmB was efficient in amounts 1000 times lower than the toxic dose.

Another antifungal agent, chloroquine, loaded into phosphatidylserine-containing negatively charged liposomes was used in MP-mediated delivery against *C. neoformans* infection in the mouse brain [107]. Administration of chloroquine-loaded liposomes accumulated inside macrophage phagolysosomes resulted in a remarkable reduction in fungal load in the brain even in low doses compared to free drug in high doses thus increasing antifungal activity of macrophages. Therefore, this drug-delivery method is effective for the transport of water-insoluble substances, such as AmB, and this warrants consideration for further testing.

6. Epilepsy

Relatively straightforward method of engineered adenosine-releasing cells was developed for treatment of epilepsy [108]. Lack of adenosine, a modulator of neuronal activity with anticonvulsant and neuroprotective properties, was found to contribute to ictogenesis. Therefore, focal reconstitution of adenosine within an epileptogenic brain region constitutes a rational therapeutic approach, whereas systemic augmentation of adenosine is precluded by side effects. To this end, human mesenchymal stem cells and human embryonic stem cells were embedded in a cell-encapsulation device to release adenosine and reduce acute injury and seizures, as well as chronic seizures [108]. Human embryonic stem cells (hESCs) have a high proliferative capacity and can be subjected to specific cellular differentiation pathways. hESCs, differentiated *in vitro* into neuroepithelial cells and grafted into the mouse brain, displayed intrahippocampal location and neuronal morphology. Overall, this therapeutic approach demonstrated antiepileptic and neuroprotective properties when grafted into the mouse hippocampus. The therapeutic potential of this approach suggests the feasibility to engineer autologous adenosine-releasing stem cells derived from a patient [109].

7. Expert Opinion

The concept of using cells as drug delivery vehicles is under development. Challenges include sufficient loading capacity and triggered drug release, preservation of therapeutic agents against degradation inside the cells, protection of cell-carriers from drug cytotoxic effects, and efficient homing of cells to the disease site. In addition, harvesting of cells sufficient quantities or their expansion is also important issue.

Two different approaches are utilized in cell-mediated drug delivery. *First*, cell-carriers (predominantly MP) are genetically modified to produce therapeutically active molecules. *Second*, host cells are loaded with a drug usually incorporated into protective container, and then used as "Trojan horses" to deliver the drug to the disease side (Figure 8). Most of cell-based delivery systems suggest the unloading the therapeutic agent at the disease site. However, a few utilized another approach, when the targeted cells accumulate the carrier along with the drug (mini-cells with anticancer agents and red blood cells with antifungal agents).

Regarding the first approach, the ideal cell delivery vehicle would be stable in tissue culture and capable of sustained, preferably regulated expression of therapeutic molecules. The cells should have appropriate and predictable differentiation pattern and survive long time *in vivo* after transplantation. Obviously, for both approaches, the cell carriers should demonstrate responsiveness to the chemotactic signals produced by the type of pathology that they are used to treat. Regarding the second approach, loading cell-carriers with drug in the blood stream by injecting nanoparticlular drug targeted to these cells is the most attractive strategy. To this end, it is important to note that these cells are moving targets. Nanosystems that bind to these targets are potentially a powerful approach for imaging their movement and delivering a therapeutic drug dose to the disease site. Clearly, a multifunctional molecular scaffold that is aimed at delivery of drug specifically to the cell-carrier, but in addition could be engineered for imaging purposes would offer substantial benefits over current approaches.

Such strategy is opposite to the common approach when drug-loaded nanoparticles are designed to minimize their entrapment in reticulo-endothelail system and avoid drug decomposition and clearance. Consequently, design of nanocarriers for cell-mediated drug delivery (size, shape, surface charge, etc.) may differ from those used for conventional drug delivery systems. Nevertheless, engaging different defense mechanisms into drug delivery may open a new perspective of active delivery of drugs for treatment of various devastating diseases.

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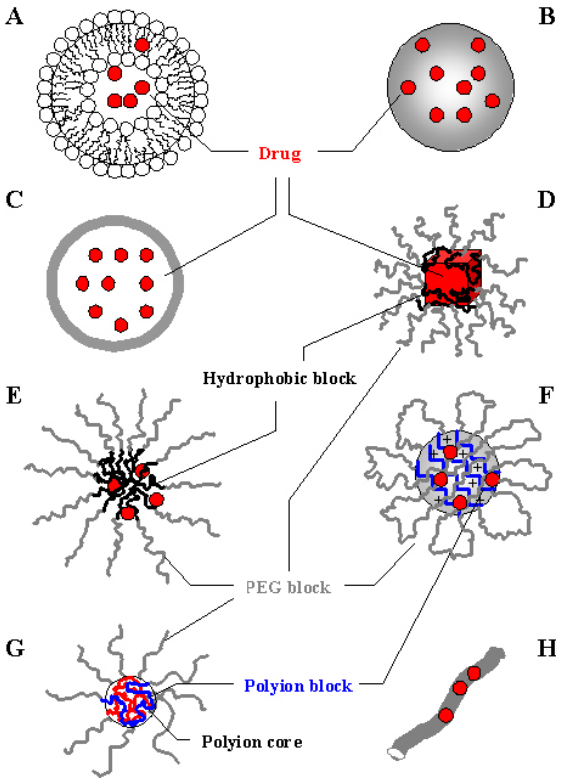

Figure 1.
Types of nanocarriers for drug delivery. **A**: liposomes; B: polymer and lipid nanoparticles; **C**: nanospheres and nanocapsules; **D**: nanosuspensions; **E**: polymer micelles; **F**: nanogels; **G**: block ionomer complexes; **H**: nanofibers and nanotubes. Used with permission [110].

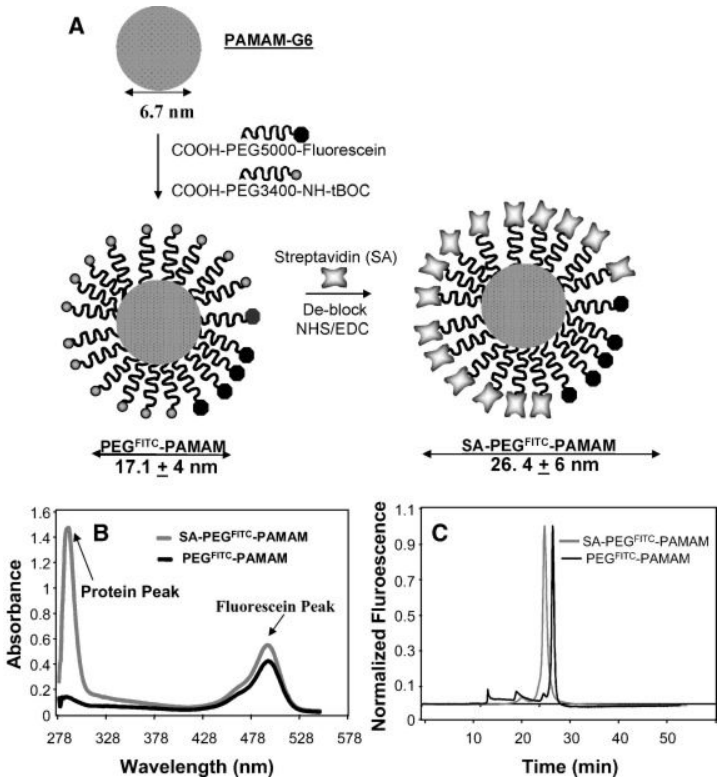

Figure 2. Schematic representation of SA-PEG^{FITC}/PAMAM construction

A. The construction of streptavidin-PEG^{FITC}/PAMAM. **B.** Absorbance profiles of streptavidin-PEG^{FITC}/PAMAM and PEG^{FITC}/PAMAM. **C**. Reverse-phase HPLC of SAPEG^{FITC}/PAMAM and PEG^{FITC}/PAMAM. Used with permission [39].

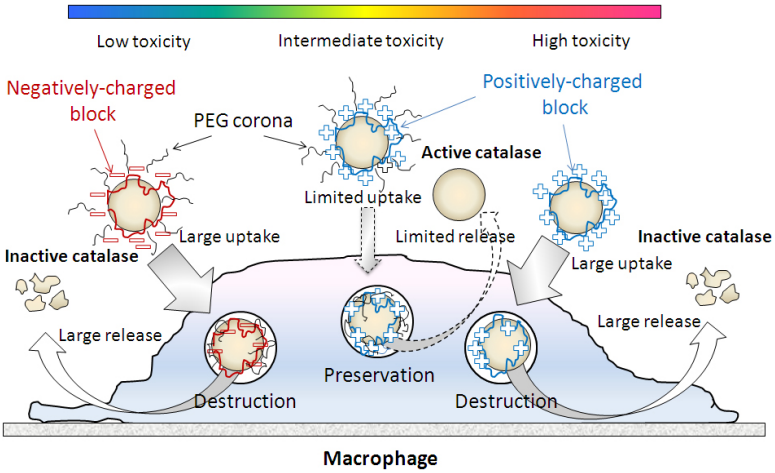

Figure 3. Optimization of nanocarriers structure for catalase delivery in macrophages

Nanozymes consisting of catalase and a negatively charged block copolymer demonstrate low toxicity, high loading capacity and release from BMM, however provide limited protection of the enzyme against protease degradation inside the cell-carriers. Nanozymes based on positively charged block copolymers, especially the PLs, showed increased cytotoxicity and low loading and release rates, but were highly protective of catalase. Furthermore, nanozymes with PEG corona show good stability in water, limited cytoxicity and efficient protection of catalase, but decreased loading capacity and cell release. The optimal nanozyme formulation is one based on positively charged block copolymers (PEI-PEG/catalase and PL-PEG/catalase). Used with permission [29].

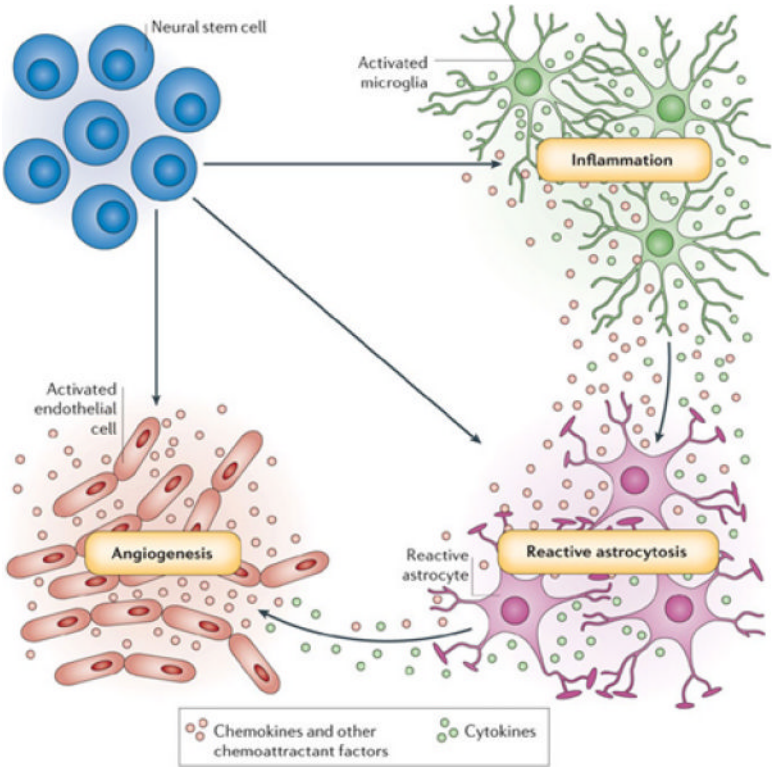

Figure 4. Using neural stem cells for drug delivery to CNS

Neural stem cells are attracted by at least three physiological processes that are common to many brain pathologies: inflammation, reactive astrocytosis and angiogenesis. Pathology-induced CNS inflammation is mediated by activated microglia that release cytokines and chemokines, which, in turn, increases the inflammatory reaction. The brain lesion and subsequent inflammation trigger reactive astrocytosis. The lesion-induced angiogenesis and inflammation-activated endothelial cells enhance neural stem cell homing to brain pathology by secreting chemoattractant factors, and also offer an atypical, perivascular niche for support of immigrating neural stem cells. Used with permission [66].

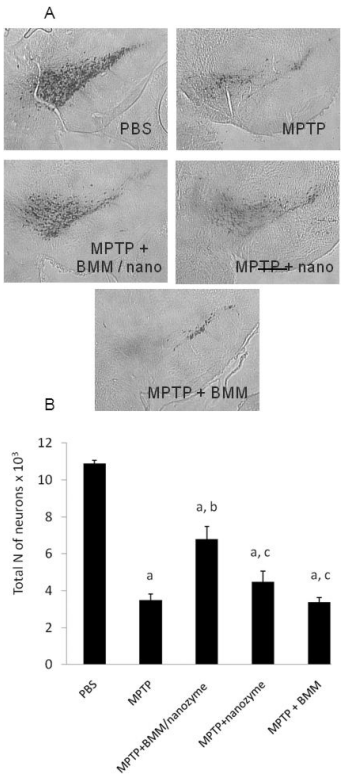

Figure 5. Neuroprotective effect of nanozyme-loaded BMM in an MPTP model of PD

MPTP-intoxicated C57Bl/6 mice (18 mg/kg) were intravenously injected with saline (second bar), nanozyme alone (third bar), macrophages loaded with nanozyme (5×10^6 cells/mouse/100μl) (fourth bar), or empty macrophages (fifth bar). Healthy non-intoxicated animals were used as control group (first bar). Seven days after treatment the animals were sacrificed; brain slices were stained for tyrosine hydroxylase-positive nigral dopaminergic neurons (**A**). Results from N = 5 animals per group demonstrate a significant loss of nigrostratial neurons in MPTP treated mice, which is prevented by adoptive transfer of macrophages loaded with nanozyme (**B**). No significant neuroprotective effect was detected after treatment with nanozyme alone, or empty macrophages. Values are means ± SEM, and $P < 0.05$ compared with [a]saline; [b]MPTP; [c]MPTP+macrophage/nanozyme. Used with permission [71].

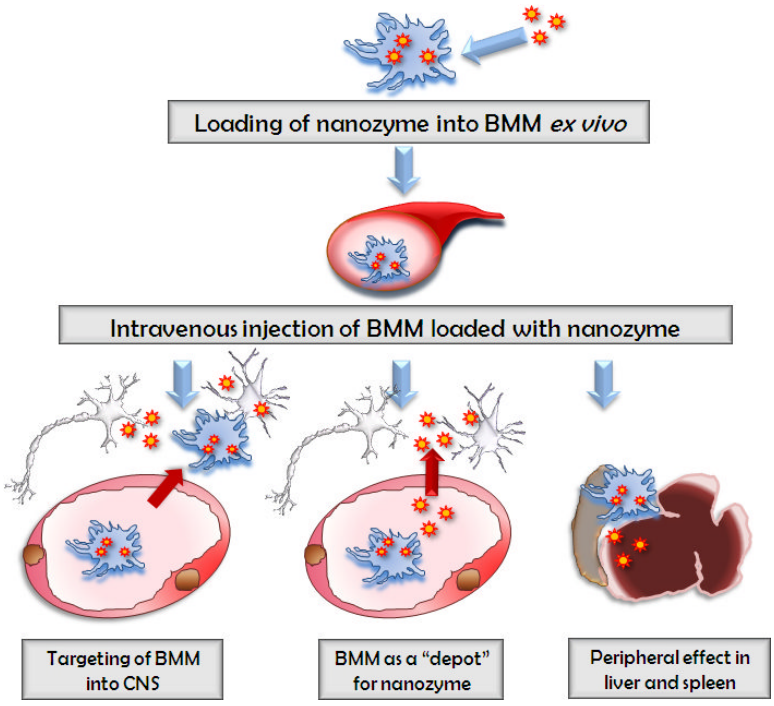

Figure 6. A pictorial scheme for cell-based nanoformulated drug delivery

Three possible ways of BMM-mediated therapeutic effects of catalase nanozyme in PD mouse model: **Pathway I**: BMM loaded with nanozyme cross the BBB and release catalase in the SNpc; **Pathway II:** nanozyme is released from BMM to the blood stream and bypasses the BBB independently of cell-carriers; **Pathway III:** catalase nanozyme released from BMM in the liver and spleen suppresses peripheral leukocyte activation that results in significant protection of nigrostratial neurons against MPTP-induced neurodegeneration. Used with permission [71].

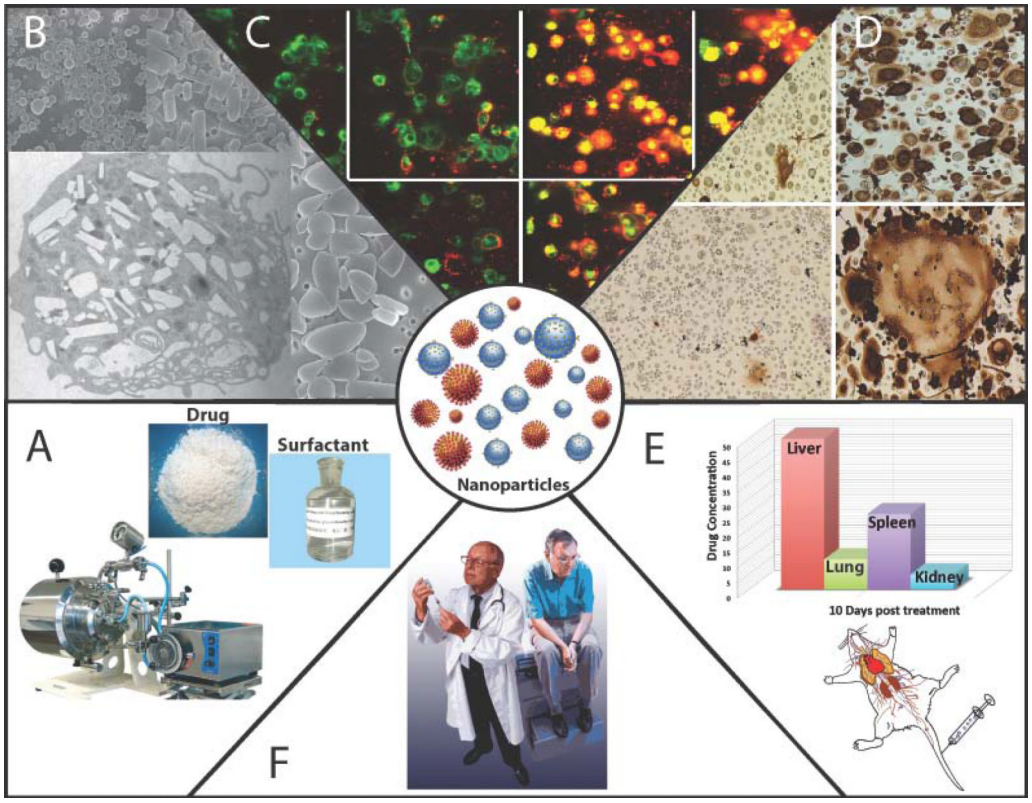

Figure 7. NanoART

(A) NanoART of antiretroviral agents were prepared by high-pressure homogenization using an AvestinC-5 homogenizer; (**B**) scanning-electron microscopy demonstrated different nanoparticle morphology; (**C**) uptake of drug-loaded nanoART (red) in monocyte-derived macrophages (green); (**D**) cells treated with nanoART showed complete or near complete suppression of HIV1 p24 antigen production; (**E**) biodistribution of nanoART in mice following intravenious injections; (**F**) patient treatment with nanoART in clinical settings. Used with permission [111].

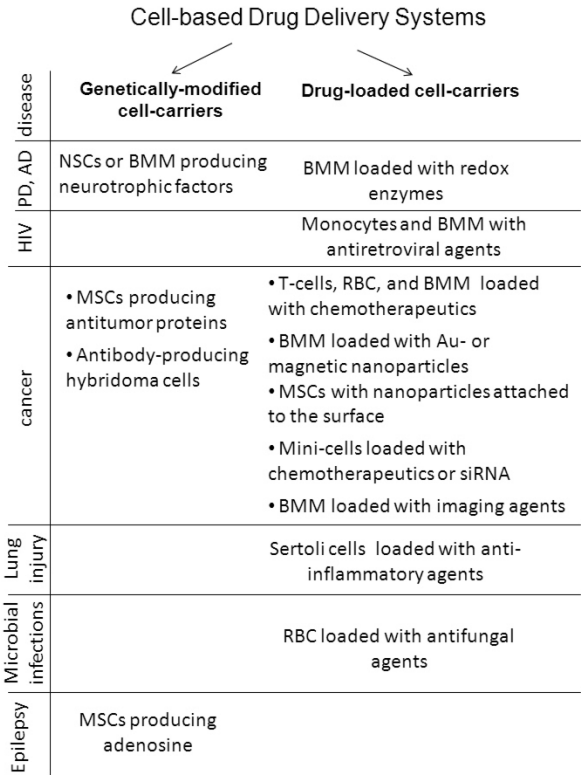

Figure 8.
Cell-based Drug Delivery Systems.

Table 1

Examples of cell-mediated drug delivery systems

Type of cell-carrier	Therapeutic agent	Rote of administration[1]	References
Alziemer's disease			
Neural stem cells (NSCs)	ciliary neurotrophic factor (NTFs)	*i.c.*	73
NSCs	nerve growth factor (NGF)	*i.c.*	65
NSCs	brain-derived neurotropic factor (BDNF)	*i.c.*	64, 75
NSCs	choline acetyltransferase (ChAT)	*i.c.*	72, 74
Parkinson's disease			
Monocytes, macrophages	catalase	*i.v.*	29, 48, 71
Fetal dopamine neurons	dopamine	*i.c.*	76
NSCs	glial cell-line derived neurotropic factor (GDNF)	*i.c.*	77, 80
NSCs	vascular endothelial growth factor (VGEF)	*i.c.*	78, 79
HIV-1 infection and neuroAIDS			
Monocytes, macrophages	atazanavir, ritonavir, indinavir	*i.v.*	14, 81-83
Cancer			
T-cells	doxorubicin	*In vitro*	94
Mesenchymal stem cells (MSCs)	tumor necrosis factor-related apoptosis-inducing ligand (TRAIL)	*ipsilateral*	93
MSCs	interferon-β	*i.v.*	86, 90, 95
MSCs	oncolytic adenovirus	*i.v.*	91, 92
MSCs	antineoplastic agent	*In vitro, in 3D collagen gels*	46
Erythrocytes	5-fluorouracil	*i.v.*	96
Monocytes and macrophages	Au-containing nanoparticles	*In vitro, in spheroids*	85, 89
Introperitoneal macrophages	5-fluorouracil	*i.p.*	50
Bacterially-derived mini-cells	small interfering RNA (siRNA), cytotoxic drugs	*i.v., i.t.*	97, 98
Bacterially-derived mini-cells	doxorubicin	*i.v., i.t.*	98
Macrophages	imaging agents, quantum dots	*i.v.*	103
Macrophages	*N*-(2-hydroxypropyl)methacrylamide (HPMA) copolymer-gadolinium (Gd) chelates	*i.v.*	104
Hybridoma cell lines 2A and OX86	anti-CD137 and anti-OX40 mAb	*s.c.*	102
Inflammation			
Macrophages	betamethasone phosphate	*in situ*	25
Erythrocytes	glycoprotein A		25
Sertoli cells	anti-inflammatory agent, curcumin	*i.v.*	105
Microbial Infections			
Erythrocytes	antifungal agent, amphotericin B	*in viro*	106
Macrophages	chloroquine	*i.v.*	107

Type of cell-carrier	Therapeutic agent	Rote of administration[1]	References
Epilepsy			
Human MSCs and human embryonic stem cells	adenosine	*i.c. in* cell-encapsulation device	108, 109

[1] *i.c.*, intracranial; *i.v.*, intravenous; *i.p.*, intraperitoneal; *i.t.*, intratumoral; *s.c.*, subcutaneous injections.