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Potential of polymeric nanoparticles in AIDS treatment and prevention

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Importance of the field: Acquired immunodeficiency syndrome (AIDS) remains one of the greatest challenges in public health. The AIDS virus is now responsible for > 2.5 million new infections worldwide each year. Despite significant advances in understanding the mechanism of viral infection and identifying effective treatment approaches, the search for optimum treatment strategies for AIDS remains a major challenge. Recent advances in the field of drug delivery have provided evidence that engineered nanosystems may contribute to the enhancement of current antiretroviral therapy.

Areas covered in this review: This review describes the potential of polymeric nanoparticle-based drug delivery systems in the future treatment of AIDS. Polymeric nanoparticles have been developed to improve physicochemical drug characteristics (by increasing drug solubility and stability), to achieve sustained drug release profile, to provide targeting to the cellular and anatomic human immunodeficiency virus (HIV) latent reservoirs and to be applied as an adjuvant in anti-HIV vaccine formulations.

What the reader will gain: The insight that will be gained is knowledge about the progress in the development of polymeric nanoparticle-based drug delivery systems for antiretroviral drugs as alternative for AIDS treatment and prevention.

Take home message: The advances in the field of targeted drug delivery can result in more efficient strategies for AIDS treatment and prevention.

Keywords: AIDS, antiretroviral drugs, polymeric nanoparticles, targeting

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1. Introduction to HIV/AIDS

Since its discovery in 1981, acquired immunodeficiency syndrome (AIDS) has remained one of the greatest public health challenges. It is now responsible for > 2.5 million new infections worldwide each year. Although progress has been made in lowering the number of AIDS-related deaths annually, the number of people living with human immunodeficiency virus (HIV) continues to grow, and programs have aimed to increase access to antiretroviral (ARV) therapy [1].

There are at present two known species of HIV: HIV-1 and HIV-2. Each species has related subspecies. HIV-1 is the most common infection globally, whereas HIV-2 is more prevalent in West Africa and has a longer latency period than HIV-1. HIV infects target cells (macrophages and T lymphocytes) by attaching its gp120 protein to the cell-surface receptor $CD4^+$. After binding, gp120 is induced to change its conformation exposing sites for chemokine co-receptors (CXCR4 or CCR5), which act as cofactors for HIV entry. After viral entry into the cell, the RNA-containing viral core is disassembled. Through the use of reverse transcriptase, the viral RNA is transcribed into DNA, which migrates to the cell nucleus where it

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Article highlights.

- AIDS continues to be a major global health priority. Although important progress has been achieved in preventing new HIV infections and the development of new drugs, the number of people living with HIV continues to increase.
- Despite the presence of highly active antiretroviral treatment, latently infected cells can persist for long periods of time and constitute 'viral sanctuaries'.
- The research involving the applications of polymeric nanoparticles in AIDS treatment and prevention has generated several interesting results.
- Passive and active targeting of antiretroviral drugs to monocytes/macrophages, an important viral cellular reservoir, can be achieved using polymeric nanoparticles.
- The brain represents an important viral anatomic reservoir. The polymeric nanoparticles are able to transpose the blood–brain barrier by well-characterized pathways.
- The use of polymeric nanoparticles as vaccine adjuvants has been explored recently, and the results show the potential of its application in anti-HIV vaccine formulation. The polymeric nanoparticles are able to increase the cellular and humoral immune response.

This box summarizes key points contained in the article.

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is inserted in the host chromosomal DNA by viral integrase. The cell is now capable of producing provirus. Viral proteins are produced and following proteolysis mediated by protease enzyme, individual viral proteins are formed and become functional. The HIV subunits combine to make up the content of the new virus. Assembly of the viral core and coat in the host cell cytoplasm leads to the maturation of the virus, which is ready to be expelled out of the host cell and infects other cells without causing host cell lysis [2].

HIV infection is characterized by a progressive loss of $CD4^+$ T cells, and this loss normally leads to severe immunodeficiency. Following infection with HIV-1, most individuals present a mononucleosis-like syndrome, a transient drop in $CD4^+$ cells, and a subsequent recovery of these initially reduced $CD4^+$ T cells to near normal levels [3]. Continuous HIV replication results in a state of generalized immune activation, and new T cells are continuously produced by the thymus to replace the ones that are lost [4]. This scenario may remain for years until the $CD4^+$ T-cell count is sufficiently low, leading to AIDS. The period from initial infection to the development of clinical AIDS can vary among individuals based on the interactions between the virus and host factors [5]. HIV causes AIDS by depleting $CD4^+$ T-helper lymphocytes that are essential to the immune response. T-helper lymphocytes prevent opportunistic infections [6]. Predicting the timing of HIV-1 disease progression requires the viral load set point because infected subjects with high levels of viremia usually progress to AIDS faster than those with lower viral load [7].

Some cell types, including macrophages and resting $CD4^+$ T cells, are recognized as potential reservoirs for HIV-1. Macrophages represent a latently infected viral reservoir, and they are a significant and critical HIV-1 target cell *in vivo*. Macrophages can be divided into multiple subsets of macrophage-like cells, all of which are susceptible to HIV-1 infection, including dendritic cells (DCs), Langerhans cells, alveolar macrophages, mucosal macrophages and microglial cells. The main anatomic reservoir sites for HIV include the lymphoid organs (particularly the spleen, lymph nodes, and gut-associated lymphoid tissue (GALT)) and the central nervous system (CNS). Undoubtedly, it is of particular importance that HIV persistence remains a serious challenge in the development of efficacious ARV therapy [8,9].

2. Current chemotherapy and drug targeting approaches in AIDS therapy

ARV treatment aims to reduce HIV replication in infected patients. The current clinical therapy, known as 'highly active antiretroviral treatment' (HAART), is considered to be one of the most significant advances in the field of HIV therapy. Since the mid-1990s, HAART has made a remarkable contribution towards reducing mortality [10].

HAART, however, is unable to eliminate HIV-1 from resting $CD4^+$ T cells in the blood because HIV persists in latency at multiple sites [11]. Despite successful administration of HAART, latently infected cells can escape the viral immune response and persist for long periods of time. Given the appropriate stimulus, latently infected cells can reactivate and start producing infectious virions. Owing to their long lifespan of several years and their ability to reactivate on encounter with cognate antigen or other stimulation, memory $CD4^+$ T cells are considered a critical reservoir for latent HIV-1 proviral DNA. Cells of the monocyte–macrophage lineage (Mo/Mac), which originate in the bone marrow, are of particular importance in HIV-1 persistence owing to their ability to cross the blood–brain barrier (BBB) and spread HIV-1 infection into the immunoprivileged CNS. Hematopoietic progenitor cells (HPCs) are also a potential HIV-1 reservoir; proviral DNA was found in HPCs *in vivo* in a subpopulation of HIV-1-infected patients. The ability of HPCs to proliferate and potentially generate clonal populations of infected cells of the Mo/Mac lineage may be crucial in HIV-1 dissemination [12].

A total of 25 compounds have been officially approved for the treatment of AIDS. At present, the different ARV drugs are classified under categories that include nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs) and, more recently, fusion and entry inhibitors (FIs and EIs) and integrase inhibitors (IIs) [13]. **Table 1** lists the approved drugs within the different classes, the available dosage forms, the main side effects and related references [14-31]. **Table 2** describes some pharmacokinetic parameters of ARV treatments and related references [32-34].

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Table 1. Approved ARV drugs within the different classes, the available dosage forms and the class-wide side effects.

Class of drug	Name of drug	Commercial name/ company	FDA approval	Dosage form	Class-wide side effects	Ref.
NRTIs	Zidovudine	Retrovir®/GlaxoSmithKline	1987	Tablets, capsules, oral solution	Lactic acidosis, hepatic steatosis, peripheral neuropathy, pancreatitis and lipoatrophy	[14-18]
	Lamivudine	Epivir®, Zeffix®/ GlaxoSmithKline	1995	Tablets, oral solution	Zidovudine-related anemia and abacavir-induced hypersensitivity reactions are common	
	Didanosine	Videx®, Videx® EC/ Bristol-Myers Squibb	1991	Tablets, capsules, oral solution, oral suspension		
	Zalcitabine	Hivid®/Roche	1992	Tablets		
	Stavudine	Zerit®/Bristol-Myers Squibb	1994	Capsules, oral solution		
	Abacavir	Ziagen®/GlaxoSmithKline	1998	Tablets, oral solution		
	Emtricitabine	Emtriva®/Gilead Sciences	2003	Capsules, oral solution		
NtRTIs	Tenofovir	Viread®/Gilead Sciences	2001	Tablets	Nephrotoxicity and osteopenia	[19-21]
NNRTIs	Nevirapine	Viramune®/Boehringer Ingelheim	1996	Tablets, oral suspension	Hepatotoxicity and skin reactions. Efavirenz is potentially teratogenic and has its own unique CNS reactions	[22,23]
	Delavirdine	Rescriptor®/Pfizer	1997	Tablets		
	Efavirenz	Sustiva®, Stocrin®/ Bristol-Myers Squibb	1998	Tablets, capsules, oral solution		
	Etravirine	Intelence®/Tibotec	2008	Tablets		
PIs	Saquinavir	Fortovase®/Roche	1995	Capsules, tablets	Severe gastrointestinal symptoms, hepatotoxicity, paresthesias, insulin resistance and hyperlipidemia	[24-28]
	Indinavir	Crixivan®/Merck	1996	Capsules	Indinavir-related crystalluria and nephrolithiasis can occur	
	Ritonavir	Norvir®/Abbott	1996	Capsules, oral solution		
	Lopinavir	Kaletra®/Abbott	1997	Capsules (with ritonavir)		
	Nelfinavir	Viracept®/Pfizer	1997	Tablets, oral powder		
	Amprenavir	Agenerase®, Prozei®/ GlaxoSmithKline	1999	Capsules, oral solution		
	Fosamprenavir	Lexiva®/GlaxoSmithKline	2003	Tablets, oral solution		
	Atazanavir	Reyataz®/Bristol-Myers Squibb	2003	Capsules		
	Tipranavir	Aptivus®/Boehringer Ingelheim	2005	Capsules, oral solution		
	Darunavir	Prezista®/Tibotec	2006	Tablets		
IIs	Raltegravir	Isentress®/Merck	2007	Tablets	Diarrhea, nausea and headache	[29]
FIs	Enfuvirtide	Fuzeon®/Roche	2003	Powder for injectable solution	The major side effects are related to injection site inflammation: induration, pruritus, nodule formation, and erythema	[30]
EIs	Maraviroc	Selzentry®, Celsentri®/Pfizer	2007	Tablets	Cough, fever, upper respiratory tract infections, rash, muscle and joint pain, abdominal pain, and postural hypotension	[31]

ARV: Antiretroviral; EIs: Entry inhibitors; FIs: Fusion inhibitors; IIs: Integrase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; NRTIs: Nucleoside reverse transcriptase inhibitors; NtRTIs: Nucleotide reverse transcriptase inhibitors; PIs: Protease inhibitors.

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Table 2. Pharmacokinetic profile of main ARV drugs [32-34].

Class of drug	Name of drug	Half-life (h)	Plasma protein binding (%)	Bioavailability (%)
NRTIs	Zidovudine	1.1	< 38	60
	Lamivudine	3 – 6	< 36	86
	Didanosine	1.3 – 1.6	< 5	30 – 40
	Zalcitabine	1 – 3	< 4	85
	Stavudine	1 – 1.6	Negligible	80
	Abacavir	1 – 2	50	83 – 100
	Emtricitabine	10	< 4	93
NtRTIs	Tenofovir	17	> 7	25 – 39
NNRTIs	Nevirapine	25 – 30	60	> 90
	Delavirdine	5.8	98	85
	Efavirenz	40 – 50	96 – 99	42 – 80
	Etravirine	30 – 40	99.9	Unknown
PIs	Saquinavir	1.5 – 2	97	Erratic
	Indinavir	1.2 – 2	60	65
	Ritonavir	3 – 5	98 – 99	65
	Lopinavir	5 – 6	98 – 99	Unknown
	Nelfinavir	3.5 – 5	> 98	20 – 80
	Amprenavir	7 – 10	90	Unknown
	Fosamprenavir	7.7	90	Unknown
	Atazanavir	7	86	Unknown
	Tipranavir	5 – 6	99.9	Unknown
	Darunavir	15	95	37
IIs	Raltegravir	9	83	Unknown
FIs	Enfuvirtide	3.8	92	84
EIs	Maraviroc	14 – 18	76	23 – 33

ARV: Antiretroviral; EIs: Entry inhibitors; FIs: Fusion inhibitors; IIs: Integrase inhibitors; NNRTIs: Non-nucleoside reverse transcriptase inhibitors; NRTIs: Nucleoside reverse transcriptase inhibitors; NtRTIs: Nucleotide reverse transcriptase inhibitors; PIs: Protease inhibitors.

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ARV drugs in the NRTI class need to be activated intracellularly in the triphosphate form to compete with endogenous deoxynucleotide-triphosphate, an essential substrate for proviral DNA, and to inhibit the activity of the viral reverse transcriptase. The drug derivates (the monophosphate form) act as chain terminators during the synthesis of proviral DNA. Seven NRTIs have been approved for use: zidovudine, lamivudine, didanosine, zalcitabine, stavudine, abacavir and emtricitabine. Zidovudine was the first ARV treatment developed; it was far from ideal, according to De Clerq [13], but it launched the search for new compounds with increased potency and safety, thereby divulging new targets and options for therapy. In general, NRTIs have limited stability, first-pass metabolism and systemic toxicity. For example, didanosine has poor stability in the gastric environment and low bioavailability owing to hepatic first pass. Zidovudine has a short half-life and hematological toxicity that is dose-dependent. Also, zidovudine and didanosine have variable bioavailability. Thus, NRTIs are ideal candidates for sustained drug release owing to a short half-life, which necessitates frequent doses, as well as severe dose-dependent side effects [35].

Tenofovir is the only commercially available NtRTI. The drug is available as tenofovir disoproxil fumarato, a prodrug. Tenofovir is also available in a fixed-dose combination with emtricitabine and also in a fixed-dose triple combination with emtricitabine and efavirenz, which provides a single daily dose for the treatment of HIV. Tenofovir disoproxil fumarato is hydrolyzed to tenofovir, which is then phosphorylated intracellularly to tenofovir diphosphate, the pharmacologically active form. Tenofovir diphosphate inhibits HIV reverse transcriptase by competing with endogenous deoxynucleotide-triphosphate for incorporation into viral DNA [36]. Tenofovir can cause renal toxicity, including acute renal failure, Fanconi syndrome, proteinuria or tubular necrosis [15,19].

NNRTIs include structurally dissimilar agents that are non-competitive inhibitors of HIV reverse transcriptase; because these drugs have a different mechanism of inhibition compared with nucleoside analogues, they can be combined with NRTIs to minimize resistance and provide synergistic effects [37]. NNRTIs include the following drugs: nevirapine, delavirdine, etravirirne and efavirenz. One of the most widely used NNRTIs is efavirenz. In contrast to other NNRTIs, efavirenz presents a more favorable resistance profile, and two or more mutations in the reverse transcription are required to generate high-level drug resistance [38]. The main drawbacks of efavirenz are its very low solubility (~ 3 – 9 µg/ml), which hinders administration, low absorption and limited biodistribution. Etravirine also presents low solubility, and no data are available about its bioavailability [39].

The PI class represents a significant advancement in the treatment of HIV infection. These drugs block HIV-1

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protease, a virus-specific enzyme that is essential for the maturation of the virus. Inhibition of this enzyme results in immature and defective viral particles. Ten PI drugs are now used in the clinic: saquinavir, the first to be developed, followed by indinavir, ritonavir, lopinavir, nelfinavir, amprenavir, fosamprenavir, atazanavir, tipranavir and darunavir. The main drawback of PI drugs is poor oral bioavailability because they are substrates for efflux pumps; as a result, individual dose adjustment is required because pharmacokinetic profiles depend on pharmacogenetic patterns [40]. Protease inhibitors also have limited penetration into the lymphatic system and CNS [37]. The poor passage of these drugs across the BBB is mainly attributed to permeability glycoprotein (P-gp)-mediated efflux. In humans, P-gp is also expressed on kidney cells, hepatocytes and intestinal cells. The P-gp expressed on intestinal cells is responsible for the reduced oral bioavailability of PIs [41]. Most PIs can increase plasma lipid concentrations, potentially increasing cardiovascular risk, and many have clinically relevant drug interactions, especially when low doses of ritonavir are used to boost the pharmacological profile. Most of these drugs are associated with gastrointestinal disturbance [28].

Raltegravir is representative of the class IIs drugs, which inhibit the insertion ('integration') of HIV-1 proviral DNA into the host genome. Raltegravir is indicated for combination with other ARVs in treatment-experienced adult patients with multi-drug-resistant HIV-1 strains [42].

Enfuvirtide is the first member of the FI class. It blocks the entry of HIV-1 into host cells by interfering with the process of virus–cell fusion, a pharmacologic target that is unique among available ARV medications; because of this unique mechanism of action, enfuvirtide remains active against HIV-1 clinical isolates that are resistant to all three classes of ARV medications (i.e., NRTIs, NNRTIs and PIs) [43]. Enfurvitide is expensive and generally poorly tolerated because of the need for twice-daily injections that often cause painful local subcutaneous reactions [44].

Maraviroc is the first member of the EI class of CCR5-receptor antagonists, a new class of ARV agents. It blocks the binding of HIV-1 viral gp120 to the CCR5 receptor and thus halts the conformational changes necessary for the entry of CCR5-tropic HIV-1 into CD4 cells. Given this unique mechanism of action, cross-resistance with other ARV classes is unlikely and has not been reported [45].

HAART comprises the combined use of three or more anti-HIV drugs. **Table 3** contains the main fixed-dose drug combination regimen recommended at present. The objectives of combined therapy are: i) to achieve synergistic activity; ii) to reduce the individual drug dose levels (and associated drug toxicity); and iii) to reduce the risk for drug resistance development [46].

HAART is highly necessary for infected patients. The discontinuation of HAART allows viral relapse from latent reservoirs. Regardless of the remarkable progress made in ARV pharmacotherapy, current HAART cannot prevent HIV replication in some anatomic (brain, gastrointestinal tract) and intracellular (Mo/Mac, hepatocytes, DCs and Langerhans cells) sites where the drugs have restricted access and short residence time [47]. Thus, the administration of higher doses is required, but is often associated with the appearance of resistance. In addition, there are also other important issues, such as adverse drug effects, poor drug regimen compliance and drug–drug interactions associated with ARV therapy [48].

Nanotechnology represents an important strategy to improve drug efficacy and reduce toxicity. Owing to the nanometer size and high surface area of the nanovectors, the pharmacokinetics of the delivered molecule can be altered. Nanocarriers have several important advantages that can improve treatment with ARV drugs, including: i) improved solubility of hydrophobic drugs; ii) increased drug bioavailability; iii) improved drug stability under physiological conditions; iv) surface characteristics that ensure uptake by phagocytic cells; and v) a more specific drug delivery and targeting strategy. This review presents the main strategies using polymeric nanoparticles for AIDS treatment and prevention.

3. Strategies using polymeric nanoparticles for AIDS therapy and prevention

Nanoparticles are solid, colloidal particles consisting of macromolecular substances varying in size from 10 to 1000 nm. A drug can be dissolved, entrapped, adsorbed, attached or encapsulated into a nanoparticle. Depending on the method of preparation, nanospheres or nanocapsules can be developed with different properties, and different release characteristics for the encapsulated therapeutic agent can also be developed. Nanocapsules are vesicular systems in which the drug is confined to a cavity surrounded by a unique polymer membrane, whereas nanospheres are matrix systems in which the drug is physically and uniformly dispersed in the polymeric matrix [49-51]. For nearly three decades, polymeric nanoparticles have been studied extensively because of their unique and valuable physicochemical and biological properties. Indeed, nanoparticles can protect the drug from degradation (physical stability during storage and in biological fluids), enhance its transport and distribution (possibility of drug targeting by modification of surface charge with inserted ligands, such as antibodies, surfactants, polymers and others) and prolong its release (ability to sustain the drug release over a period of days to weeks); therefore, they may improve the plasma half-life of the drug entrapped [52,53]. The pharmacokinetic parameters are altered with the nanoparticles, and their surface composition plays an important role in drug bioavailability, which can be greater or lower than the drug solution/powder ratio depending on the polymer used [54,55]. As some nanoparticle characteristics, such as particle size and surface charge, can be modulated by modifying some process parameters on their formulation, they can be used in various applications involving different routes of administration.

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Table 3. The main fixed-dose drug combination regimen anti-HIV recommended at present.

Fixed-dose drug combination regimen	Drugs	Commercial name/company	FDA approval	Dosage form
Double combination	Lamivudine/zidovudine	Combivir®/GlaxoSmithKline	1997	Tablets
	Abacavir/lamivudina	Epzicom®/GlaxoSmithKline	2004	Tablets
	Tenofovir/emtricitabine	Truvada®/Gilead Sciences	2004	Tablets
	Lopinavir/ritonavir	Kaletra®/Abbott	2000	Tablets, oral solution
Triple combination	Abacavir, lamivudine and zidovudine	Trizivir®/GlaxoSmithKline	2000	Tablets
	Tenofovir/emtricitabine/ efavirenz	Atripla®/Gilead Sciences and Bristol-Myers Squibb	2006	Tablets

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Although polymers are the most widely used materials, nanoparticles consist of a variety of materials, including polymers, proteins and lipids. The polymers used include natural and synthetic materials, and the main characteristics required are biodegradability and biocompatibility. In general, synthetic polymers (polyesters and their copolymers polyacrylates and polycaprolactones) offer greater advantages than natural ones (albumin, gelatin, alginate, collagen and chitosan) in that they can be tailored to have a wider range of properties. Natural polymers have not been widely used because they vary in purity, and they often require crosslinking, which could denature the embedded drug [56-58]. The general criteria for selecting a polymer for use as a degradable biomaterial are to match the mechanical properties and the degradation rate to the needs of the application.

The advantage of using polymeric nanoparticles as colloidal carriers for advanced drug delivery is mainly their small size, which allows nanoparticles to penetrate into even small capillaries and be taken up within cells, allowing efficient drug accumulation at targeted sites in the body. Also, the biodegradable polymers used for their preparation allow for sustained drug release at the targeted site over a period of days or even weeks after administration [59].

The research involving the applications of polymeric nanoparticles in AIDS treatment and prevention has generated several important results and demonstrates its potential for this objective. The main strategies discussed in this review are based on: i) the intracellular delivery of ARV drugs by polymeric nanoparticles; ii) brain delivery of ARV drugs by using polymeric nanoparticles; and iii) the use of polymeric nanoparticles as an adjuvant for anti-HIV vaccines.

3.1 Intracellular delivery of ARV drugs by polymeric nanoparticles

Mo/Mac cells are widely recognized as the secondary cellular target of HIV-1 and a crucial virus reservoir [60]. HIV-1-infected Mo/Mac cells are widely distributed in all tissues and organs, including the CNS, where they represent the majority of cells infected by HIV-1 [61,62]. HIV-1 replication in Mo/Mac is a crucial pathogenic event during the progression of viral infection. HIV-1 infection in Mo/Mac is characterized by viral dynamics substantially different from that of $CD4^+$ lymphocytes. In fact, activated $CD4^+$ lymphocytes can sustain a rapid and exponential viral production followed by massive cell death [63]. By contrast, Mo/Mac are resistant to the cytopathic effect of HIV-1 and produce virus over a prolonged period; production increases linearly during the first 1 – 2 weeks of infection, followed by a plateau of the high level of replication (> 10^8 copies of unspliced/spliced RNA produced) lasting at least up to 60 days after infection [64]. Mo/Mac can survive HIV-1 infection for long periods of time. This is mainly related to the autocrine secretion of the nerve growth factor (NGF) associated with enhanced expression of the high affinity NGF receptor p140 trkA on the Mo/Mac surface. This complex interaction enhances the ability of macrophages to cope with HIV infection, thus transforming them into a long-term viral reservoir [65]. Plus, these cells are implicated in HIV transmission routes, including maternal–fetal [66], mucosal [67] and sexual transmission [68]. Based on these facts, it is obvious that Mo/Mac represents an important target for ARV drugs and for carriers loaded with these drugs.

When administered intravenously, conventional colloidal carriers are rapidly cleared from the bloodstream by the mononuclear phagocyte system (MPS), represented by monocytes and macrophages. This removal from the circulation generally occurs through recognition by cellular receptors specific for plasma proteins bound to the carriers rather than recognition of the carriers themselves [69]. The recognition of particles by macrophages is mediated by a process called opsonization, which depends on the distance between the particles and opsonins. When this distance is sufficiently small, the opsonins bind to the surface of particles and become recognizable to macrophages, and phagocytosis occurs [70]. It is well known that particle uptake by cells is affected by the particle's physicochemical properties, such as particle size, surface charge, hydrophobicity and presence of a coating (varying in density/conformation). A correlation between surface charge and phagocytosis has been demonstrated *in vitro*; the uptake of charged particles occurs more quickly and to a greater extent than the uptake of neutral particles [71]. Based on this knowledge, the development of an ARV carrier system

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intended for targeting Mo/Mac is an attractive concept in AIDS treatment.

One of the first studies involving ARV treatments and macrophage targeting using nanoparticles was conducted by Schäfer and co-workers [72]. Their *ex vivo* study used macrophages isolated from the peripheral blood of HIV-infected or uninfected patients. The authors found that the physicochemical properties, including the composition, surface characteristics and size, of poly(alkyl cyanoacrylate) (PACA), poly(methyl methacrylate) (PMMA) and human serum albumin (HSA) nanoparticles containing zidovudine influenced the rate of uptake by macrophages, particularly when these cells were infected by HIV (up to 60% more than for uninfected macrophages). Thereafter, the group developed poly(hexyl cyanoacrylate) (PHCA) and HSA nanoparticles containing zidovudine and didanosina and demonstrated *in vitro* the effectiveness of these drug-targeting systems in preventing HIV infection in Mo/Mac cultures [73]. Furthermore, the group prepared PHCA nanoparticles as carriers for saquinavir (475 nm) or zalcitabina (200 nm) by an emulsion polymerization method (poloxamer 188 as stabilizer) and tested them for antiviral activity in primary human Mo/Mac *in vitro*. Both nanoparticulate formulations led to a dose-dependent reduction of HIV-1 antigen production. Whereas nanoparticle-bound zalcitabine showed no superiority compared with free drug, a significantly higher efficacy was observed with saquinavir-loaded nanoparticles. In acutely infected cells, the saquinavir aqueous solution showed little antiviral activity at concentrations < 10 nM, whereas the nanoparticulate formulation showed a good antiviral effect at a concentration of 1 nM and a significant antigen reduction at 0.1 nM (IC_{50} = 4.23 nM for free drug and 0.39 nM for nanoparticle-bound drug). At a concentration of 100 nM, saquinavir was completely inactive in chronically HIV-infected macrophages, but when bound to nanoparticles, saquinavir caused a 35% decrease in antigen production [74].

In a similar study, Shah and Amiji [75] designed saquinavir-loaded poly(ethylene oxide)-modified poly(epsilon-caprolactone) (PEO-PCL) nanoparticles (200 nm) for intracellular delivery. The saquinavir carried in the PEO-PCL nanoparticles was significantly internalized by the THP-1 human Mac/Mo cell line at a 10-fold higher rate compared with saquinavir in an aqueous solution, according to a fluorescent probe. The intracellular concentration of saquinavir, after incubation with Mo/Mac cultures, was evaluated as a function of the dose administered and incubation length. In the first case, at a 50 nM dose, the intracellular concentration of saquinavir delivered through the nanoparticle was eightfold higher in concentration than the delivery in an aqueous solution. A similar trend was observed when the cells were treated with a 50 nM dose of saquinavir for different time intervals. Microscopic observations qualitatively showed that a significantly higher percentage of the administered dose of nanoparticles was internalized by the cells.

In another study, Hillaireau *et al.* [76] developed poly(isobutyl cyanoacrylate) (PIBCA) nanocapsules as carriers for azidothymidine-triphosphate (AZT-TP) and cidofovir, and preliminary studies suggested that AZT-TP rapidly leaked out of the nanocapsules after encapsulation, which was attributed to the rapid diffusion of the molecule through the pores of the thin polymer nanocapsule wall. Furthermore, the same group developed hybrid nanocapsules consisting of PIBCA and poly(ethylene imine) to increase the encapsulation efficiency of AZT-TP. The particles were analyzed for intracellular uptake by J774.A1 cells (mouse macrophages). As expected, free AZT-TP was poorly absorbed by the cells, whereas the uptake of AZT-TP was 10 – 30-fold higher when it was delivered by nanocapsule [77].

In work from the authors' group, polylactic acid (PLA) and PLA-polyethylene glycol (PLA-PEG) blend nanoparticles containing zidovudine (265 – 374 nm) were developed, and their uptake by polymorphonuclear leukocytes from rats was studied *in vitro*. The influence of the polymer type on particle size, zeta potential and particle uptake by polymorphonuclear leukocytes was investigated. The cells were isolated from rat peritoneal exudate, and their activation by nanoparticles was measured by luminol-dependent chemiluminescence and microscopic analysis. The PEG in the blend modified the zeta potential, suggesting the formation of a PEG coat on the particle surface. The phagocytosis was dependent on the PEG and its ratio in the blend. The results showed that the PLA nanoparticles (ζ -20 mV) were more efficiently phagocytosed than PLA-PEG blends (ζ -6.5 mV). The blend with the highest PEG proportion did not prevent phagocytosis, indicating that the steric effect of PEG was concentration-dependent, owing to the conformation acquired on the nanoparticle surface. Also, the interaction between the various nanoparticle formulations and the cells was observed microscopically by counting the formation of intracellular vacuoles corresponding to nanoparticle phagocytosis. The PLA nanoparticles were observed to be able to activate a larger number of cells (51.5 ± 7.5) than the PLA-PEG blend nanoparticles (19.3 ± 6.1). Interestingly, the PLA nanoparticles were able to activate the cells more extensively, as indicated by the larger number of intracellular vacuoles formed by the cell (~ 2.2-fold) compared with those induced by the PLA-PEG blend particles (1:0.25). The nanoparticles comprising the PLA-PEG blend with the highest PEG proportion had a profile very similar to the PLA nanoparticles, and these observations confirmed the results obtained in the chemiluminescence assay [78]. Furthermore, the group evaluated the pharmacokinetic profile of the zidovudine-loaded PLA and PLA-PEG blend nanoparticles in rats after a single intranasal administration [79]. PLA-PEG blend nanoparticles showed sustained release of the drug over 24 h. The T_{max} for this formulation was increased twofold compared with zidovudine from PLA nanoparticles and 16-fold compared with the zidovudine aqueous solution. The drug levels in plasma were detectable up to 10 h after administration in the aqueous solution or PLA nanoparticles. The $t_{1/2}$ of zidovudine also varied among

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the formulations. The slow elimination rate (K_e) resulted in significantly prolonged $t_{½}$ of zidovudine from the PLA and PLA-PEG blend nanoparticles compared with the zidovudine solution. Owing to the slow release of zidovudine from the nanoparticles, its metabolic breakdown was also slower, increasing the mean half-life. The significant increase ($p < 0.05$) in the area under curve (AUC) value of the zidovudine-loaded PLA-PEG nanoparticles in comparison with that of the PLA nanoparticles and zidovudine aqueous solution distinctly indicated the improved intranasal bioavailability of the blended system. Thus, the results of this study corroborated those of the first study, indicating that the determination of physicochemical characteristics of nanoparticles intended for controlled drug release is very important because these characteristics can govern the application of the formulation and can be used to predict its behavior in the biological medium. The size and surface charge are important parameters in a nanostructured system because these characteristics interfere directly in biological processes, such as transport across biological membranes, recognition by Mo/Mac and biodistribution.

Destache and co-workers [80] developed poly(lactic-*co*-glycolic) acid (PLGA) nanoparticles containing three ARV drugs ritonavir, lopinavir and efavirenz. The nanoparticles (262 ± 83.9 nm and ζ -11.4) were developed by water-in-oil-in-water homogenization. The *in vitro* release of the ARV drugs from the nanoparticles in human peripheral blood mononuclear cells was investigated, and the results showed an intracellular peak of each drug over 28 days, whereas nanoparticle-free drugs were eliminated in 2 days. Macrophages were imaged by fluorescent microscopy and flow cytometry, and phagocytosis of nanoparticles by Mo/Mac was demonstrated. A cytotoxicity assay performed on the cells demonstrated that the nanoparticles were not significantly toxic. These results are important because they demonstrate that the three drugs can be incorporated into a single nanoparticle for drug delivery. The use of a single ARV drug in the treatment of HIV-1 only results in the development of resistant strains and treatment failures. In another recent paper, these nanoparticles were administered intraperitoneally to mice for a pharmacokinetic study. Serum-free ARV drug concentrations were observed to peak at 4 h post-injection (ritonavir 3.9 ± 3.05, lopinavir 3.4 ± 2.5 and efavirenz 1.8 ± 0.63 mg/ml) and were eliminated by 72 h. PLGA nanoparticle-administered ritonavir, lopinavir and efavirenz had detectable concentrations in all tissues for 28 days. These results showed that the ARV drugs associated with nanoparticles were able to maintain the drug concentrations for a prolonged period. In brain tissue, the peaks induced using the ARV drug-loaded nanoparticles were significantly higher than those induced using the free ARV drug. None of the three freely injected ARV drugs reached a concentration of 1 mg/g in the brain tissue during the experiment. In sharp contrast, the concentrations of the three ARV drugs delivered by nanoparticles peaked at concentrations of 5 mg/g in the brain. Indeed, lopinavir concentrations in the brain were still detectable (averaging 1.2 mg/g) 35 days post-injection of a single dose. Also, the ARV nanoparticles were able to interact with the Mo/Mac infected with HIV-1 and inhibit virus replication up to 1000-fold for 10 days compared with free drugs. These data provided evidence that the nanoparticles may be able to offer sustainable HAART [81].

The lymphoid organs (liver and spleen) contain immune cell populations, particularly macrophages, which are important targets in HIV therapy. Löbenberg and co-workers [82,83] obtained PHCA nanoparticles containing zidovudine by emulsion polymerization (bis-2-ethylhexyl sulfosuccinate sodium as stabilizer). After intravenous administration in rats, a higher drug concentration was observed in the organs of the reticuloendothelial system (RES) (liver and gastrointestinal tract); the concentration was ~ 18 times higher compared with the zidovudine solution (control). A specific nanoparticle's uptake by the macrophages was suggested because the radioactivity was higher in organs rich in macrophages [83]. Another important effect observed was the higher levels of zidovudine in the brain when the nanoparticles were coated with polysorbate 80 (PS-80) [84]. Dembri and co-workers [85] evaluated the capacity of PHCA nanospheres to concentrate zidovudine in the intestinal epithelium and associated immunocompetent cells after oral administration in rats. Unlike the nanoparticle-free drug given in solution, the nanoparticle formulation concentrated zidovudine very efficiently in the intestinal mucosa and in the Peyer's patches, and it could simultaneously control the release of free zidovudine. The concentration of the drug in the Peyer's patches was four times higher for nanoparticles compared with the control solution. The tissue concentration was 30 – 45 μM, which was much higher than the reported IC_{50} of zidovudine (0.06 – 1.36 μM), and it was regularly distributed along the gastrointestinal tract. This work supported the view that these particles may represent a promising carrier to treat specifically the gastrointestinal reservoir of HIV. The normal uptake of nanoparticles by macrophages present in the RES is indeed an important passive method for targeting this anatomic reservoir site.

HIV-targeted cells, particularly macrophages, are also able to take up particles by receptor-mediated endocytosis, increasing the cell-specific uptake. Receptor-mediated drug targeting is one of the most promising approaches to achieving efficient therapy and minimizing systemic toxicity. Subsequent internalization of the carrier–drug complex leads to the accumulation of drug in the target cells and exclusion from non-target cells that lack the requisite receptor [86].

Macrophages possess various receptors, such as fucose receptors, mannosyl, galactosyl, and many others. Mannose receptors are present at the surface of Mo/Mac, alveolar macrophages, astrocytes in the brain, hepatocytes, and so on [87,88]. These receptors help in the recognition and endocytosis of a particulate carrier. Therefore, carriers containing ligands such as mannosyl, immunoglobulin, fibronectin and galactosyl are better phagocytosed by macrophages than

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carriers without such ligands [89]. Jain *et al.* [90] developed and explored the use of mannosylated gelatin nanoparticles for the selective delivery of an anti-HIV drug didanosine to target organs. Mannosylated gelatin nanoparticles (248 – 325 nm) were prepared using a two-step desolvation technique and coupled with mannose using the amino group of gelatin present on the surface of nanoparticles. The *ex vivo* cell uptake studies that used alveolar macrophages from male albino rats showed a significant increase in cellular drug uptake when mannosylated gelatin nanoparticles were used; the use of these nanoparticles resulted in drug concentrations 18.0 and 2.7 times higher than those achieved using the free drug and uncoated gelatin nanoparticles, respectively. In a study using fluorescence microscopy, it was revealed that intravenous administration of mannosylated coated nanoparticles in rats induced a higher intensity of fluorescence in the lung tissue, lymph nodes and liver compared with that using the uncoated drugs. This occurred because mannose receptors are present in higher numbers in these organs. These receptors recognize the mannosylated gelatin nanoparticles, which are taken up by a receptor-mediated endocytosis mechanism, thus resulting in higher uptake and localization of the nanoparticles. The authors concluded that the mannose-conjugated drug delivery system with site specificity could help to reduce the toxicity of the available anti-HIV doses and formulations.

In a similar study, Kaur *et al.* [91] formulated sustained- and targeted-release nanoparticles with didanosine using gelatin as the polymer and mannan coating to enhance further its macrophage uptake and distribution in organs that are major reservoirs of HIV. The nanoparticles were prepared using the double desolvation technique (95 – 235 nm) and were evaluated *in vitro*, *ex vivo* and *in vivo*. Results of the *ex vivo* cellular uptake study indicated a fivefold higher macrophage uptake of didanosine from the mannan nanoparticle formulation compared with the didanosine solution. Results of the quantitative biodistribution study showed that the localization of didanosine in the spleen, lymph nodes and brain was 1.7, 12.6 and 12.4 times higher, respectively, after administration of mannan-coated nanoparticles compared with the nanoparticle-free didanosine solution. However, the authors did not evaluate the uncoated gelatin nanoparticles, although they did show the role of mannan in promoting the receptor-mediated endocytosis by mannosyl receptors.

The previous studies have shown that this strategy is a highly versatile alternative to modify the nanoparticles to display different structural features. Nanoparticles represent a compelling carrier system for the targeted delivery of ARV agents to macrophages.

3.2 Brain delivery of ARV drugs by polymeric nanoparticles

Considering that the CNS is an important reservoir for HIV and that the reason for therapeutic failure is multifactorial, the treatment and control of HIV within this reservoir are primordial. Owing to the restricted entry of anti-HIV drugs, the brain is thought to form a viral sanctuary. This restriction not only results in virologic resistance (generating a virus pool that curtails the total elimination of HIV), but also is regularly associated with the development of complications such as HIV-associated dementia, neuroinflammation, latent infection and neurodegeneration [92]. HIV-associated dementia is characterized by a cognitive impairment (short-term memory, reduced concentration, learning capability and psychomotor skills), motor dysfunction and behavioral changes (personality changes, apathy and social withdrawal) [93,94]. Thus, the targeting of ARV drugs to the brain has become a significant goal for drug therapy.

Current ARV therapy often fails to reduce effectively the viral load in the brain. CNS delivery of anti-HIV drugs is limited by the BBB and blood–cerebrospinal fluid (CSF) interfaces owing to a combination of restricted paracellular movement, powerful metabolic enzymes and numerous transporters, including members of the ATP binding cassette (ABC) and solute carrier (SLC) superfamilies [95]. Thus, drug delivery into the brain is limited under both normal and pathological conditions. The urgent need to develop new strategies to improve drug delivery to this tissue is evident. The use of nanoparticles for drug delivery to the brain across the BBB may provide a significant advantage over other strategies. A review by the authors' group described some important results obtained with polymeric nanoparticles in drug delivery to the brain [96].

Effective treatment of HIV infection in the brain requires long-term maintenance of therapeutic concentrations of ARV drug in the brain. This maintenance leads to sustained suppression and eventual elimination of HIV-1 in the viral reservoirs within sequestered regions of the brain. However, the systemic delivery of ARV drugs in the brain is severely hampered by the BBB. Therefore, fabrication of new carriers that would significantly enhance the delivery of drugs across the BBB holds the key for the treatment of neuro-AIDS and other neurological diseases [97].

Nanoparticles can enhance brain drug delivery by three major pathways, which are: i) increasing the local drug gradient at the BBB by passive targeting; ii) allowing drug-trafficking by nonspecific or receptor-mediated endocytosis; and iii) blocking drug efflux transporters at the BBB [98]. Consequently, the use of nanocarriers should help not only to achieve higher concentrations of encapsulated drugs, but also to allow their prolonged residence in the CNS.

One of the most used polymers for the development of nanoparticles intended for brain delivery is poly(butyl cyanoacrylate) (PBCA). These nanoparticles are prepared by polymerization techniques, with the drug adsorbed onto the surface of the nanoparticles pre-formed [99]. Studies showed that surface modification of PBCA nanoparticles using other polymers or tensoactive agents (non-ionic) could increase the transport of particles through the BBB. PS-80 has been used for this purpose, and studies denoted higher translocation of nanoparticles coated with this surfactant

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into the brain than that of uncoated PBCA nanoparticles [100]. Studies revealed that PS-80 can increase the concentration of apolipoprotein E (apoE) adsorbed on the nanoparticle surface, and these apoE-enriched nanoparticles probably exploit the low-density lipoprotein (LDL) receptor-mediated endocytic pathway in brain endothelial cells [101]. Furthermore, PS-80 could inhibit the efflux function of P-gp to promote the BBB penetration efficiency [102,103].

PBCA and methylmethacrylate-sulfopropylmethacrylate (MMSPM) nanoparticles (59 – 149 nm) containing stavudine were designed for brain targeting [104]. This work emphasized the effect of particle size on drug loading; a smaller size resulted in higher drug loading. Subsequently, Kuo and Chen [105] observed the effect of PBCA and MMSPM nanoparticles on the permeability of zidovudine and lamivudine across the BBB using the blood-brain-microvascular endothelial cells model. Drug permeability increased with the decrease in particle size of the two polymeric carriers. A better result was obtained with the MMSPM nanoparticles, which were able to increase significantly, by 100%, the BBB permeability of both drugs. PBCA nanoparticles increased the BBB permeability of zidovudine 8 – 20-fold and lamivudine 10 – 18-fold. In the follow year, these authors observed an increase in BBB permeability (*in vitro* study) of stavudine-, delaviridine- and saquinavir-loaded PBCA and MMSPM nanoparticles coated with PS-80 and also solid lipid nanoparticles, and higher drug permeability was obtained with smaller particles [106]. PS-80 had an important role in the entry of nanoparticles by means of receptor-mediated transcytosis, mimicking LDL particles. The permeability of the three drugs was enhanced ~ 12 – 16-fold with PBCA nanoparticles, 3 – 7-fold with MMSPM nanoparticles and 4 – 11-fold with solid lipid nanoparticles.

Receptor-mediated endocytosis provides a means for selective uptake of macromolecules and also small molecules. At the BBB, this process occurs for substances such as transferrin, insulin, leptin, insulin-like growth factor and LDL and is an energy-dependent transport that may be saturated. The transferrin and LDL receptors have been studied for specific targeting of drugs and drug carriers to the brain [107,108].

The transferrin receptors present in the luminal membrane of brain endothelial cells have been used as preferential targets for enhanced ARV drug delivery to the CNS by means of nanoparticulate systems [109]. Long circulatory PEGylated albumin nanoparticles encapsulating zidovudine were prepared by an ultra-emulsification method (120 nm) using chemical crosslinking by glutaraldehyde. The surface of the PEGylated nanoparticles was modified by anchoring transferrin as a ligand for brain targeting. Fluorescence studies revealed the enhanced uptake of transferrin-anchored nanoparticles in the brain tissues compared with the uptake of unmodified nanoparticles with transferrin. An *in vivo* evaluation was conducted in albino rats to evaluate the tissue distribution of engineered nanoparticles after intravenous administration. The authors observed a significant enhancement of brain localization of zidovudine when it was delivered by transferrin-anchored PEGylated albumin nanoparticles [110].

Recently, the properties of cell-penetrating peptides have been explored to enhance further the cellular permeability of drug carrier systems. In this approach, certain proteins or peptides can be tethered to the hydrophilic drug of interest, and together the construct possesses the ability to translocate across the plasma membrane and deliver the payload intracellularly [111]. The Tat peptide, the most frequently used cell-penetrating peptide, is derived from the transcriptional activator protein encoded by HIV-1 [112]. The ability of the Tat peptide to permeate biological membranes using different pathways, including clathrin-dependent endocytosis, lipid raft-dependent macropinocytosis and the use of non-endocytic pathways such as direct movement through lipid bilayers, makes it a promising system for intracellular drug delivery [113,114]. Fusion of β-galactosidase to the Tat peptide demonstrated the passage of the conjugated biomacromolecule across the BBB in mice [115]. Thus, nanoparticles containing Tat are promising systems for transport across the BBB and entry into the brain. Therefore, Rao and colleagues [116] hypothesized that anti-HIV drugs loaded in nanoparticles could bypass the efflux action of P-gp and that Tat conjugation would enhance their transport across the BBB, thereby enhancing the CNS bioavailability of anti-HIV drugs. In their study, ritonavir-loaded PLA nanoparticles conjugated with the Tat peptide were developed (300 – 340 nm) using an emulsion-solvent evaporation technique (PVA as stabilizer). They demonstrated enhanced and sustained brain delivery of ritonavir-loaded Tat-conjugated nanoparticles without influence on the integrity of the BBB, suggesting that the transport occurred through transcytosis across the endothelium of the brain vasculature. At 2 weeks post-administration, the brain ritonavir level after administration of the conjugated nanoparticles was 800-fold higher than that with the drug delivered in solution. Drug clearance was seen within 4 weeks. The authors concluded that Tat-conjugated nanoparticles enhanced the CNS bioavailability of the ritonavir and maintained therapeutic drug levels in the brain for a sustained period that could be effective in reducing the viral load in the CNS, which acts as a reservoir for the replicating HIV-1 virus.

Another way to continue the research in brain delivery is to circumvent the BBB by applying alternative routes of administration, such as the intranasal route. Intranasally administered therapeutics reach the CNS via the olfactory and trigeminal neural pathways. Both the olfactory and trigeminal nerves innervate the nasal cavity, providing a direct connection with the CNS. Direct delivery of therapeutics from the nose to the brain was initially attributed to the olfactory pathway [117,118]. More recently, the contribution made by the trigeminal pathway to intranasal delivery to the CNS has also been recognized, especially to caudal brain regions and the spinal cord [119,120]. This technology allows drugs

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that do not cross the BBB to be delivered to the CNS within minutes. It also delivers directly to the brain drugs that do cross the BBB, eliminating the need for systemic administration and its potential side effects. The Hanson and Frey review [121] cited the potential of intranasal delivery of drugs for treatment of neuro-AIDS. Intranasal administration as a potential route for enhancing brain delivery of stavudine was investigated in rats by Yang *et al.* [122] using microdialysis as a sampling technique. Following intranasal administration, stavudine was rapidly and completely absorbed into systemic circulation and had a T_{max} of 14 min and bioavailability of 105%. The half-life of stavudine in the various brain regions was significantly longer than that in the plasma.

Previous work explored the application of drug-loaded nanoparticles by the intranasal route intended for direct nose-to-brain transport. Some research showed an improvement in the brain delivery of molecules such as morphine [123] and nimodipine [124] loaded in polymeric nanoparticles and administered by the intranasal route. Until now, in the field of ARV drugs, only one study has investigated the potential use of chitosan nanoparticles as a delivery system to enhance the systemic and brain-targeting efficiency of didanosine following intranasal administration. The brain/plasma, olfactory bulb/plasma and CSF/plasma concentration ratios were significantly higher ($p < 0.05$) after intranasal administration of didanosine nanoparticles or solution than those after intravenous administration of the didanosine aqueous solution. The study showed that both the intranasal route of administration and the formulation of didanosine in chitosan nanoparticles increased the drug delivery to the CSF and brain [125]. Certainly, the exploration of the intranasal route is a promising means for drug delivery to the brain.

3.3 Polymeric nanoparticles as adjuvant for anti-HIV vaccines

The development of an HIV-1 vaccine is much needed to prevent the continuing spread of the AIDS pandemic across the world [126]. HAART has reduced the AIDS death rate and suppressed viral replication in infected individuals, but the complete eradication of the virus from infected patients by HAART does not seem possible, suggesting the necessity for long-term treatment. Moreover, the side effects and emergence of drug-resistant viruses limit the long-term application of HAART [127]. Thus, an effective, safe and affordable HIV-1 vaccine with prophylactic/therapeutic effects is most desirable for the eradication of HIV-1 infection.

Nanosystems have been studied in the development of vaccines as adjuvants. These adjuvants play an important role because in many cases the antigen itself is only very weakly immunogenic; therefore, an adjuvant is needed to intensify the immune response. Adjuvants can also be included in vaccines to guide the type of immune response generated [128]. This may be especially important when developing vaccines for cancer [129], HIV [130] or mucosal immunizations [131].

The main adjuvant licensed for human use is aluminum salt. Apart from its wide use, it presents some disadvantages, such as instability to freezing and drying [132] and inconsistencies in inducing humoral immunity [133]. Also, despite maintaining a good safety profile for more than seven decades, some safety concerns exist regarding the use of aluminum salts [134].

Thus, with very few adjuvants now being used in marketed human vaccines, a critical need exists for new immunopotentiators and delivery vehicles capable of eliciting humoral, cellular and mucosal immunity. Polymeric nanoparticles have been studied as vaccine adjuvants because of their safety profile and ability to protect labile immune-stimulating molecules (e.g., DNA or peptides/proteins) and enhance their immunogenicity when delivered by diverse routes (e.g., intramuscular, subcutaneous, intradermal and intranasal) [134-137]. Microparticle- and nanoparticle-prepared PLGA or PLA polymers represent a successful method for *in vivo* delivery of peptide, protein or DNA antigens. These polymers were developed primarily as resorbable sutures and controlled-release drug delivery systems [138], and they have an excellent safety record. Moreover, PLGA microspheres have several more advantages, such as the ability to elicit cytotoxic T-lymphocyte (CTL) responses, and the potential for mucosal immunization and DNA delivery [139,140]. Recent work by Sharp *et al.* [141] has shown that cellular internalization of PLGA and polystyrene microparticles induced large amounts of IL-1β production in DCs via inflammasome activation.

In a study by Locher *et al.* [142], an HIV-2 env DNA vaccine was synthesized and delivered in a new polycationic adjuvant formulation (consisting of polylysine as a cationic center containing imidazole side chains to optimize endosomal escape) that formed nanoparticles in solution (140 nm) and enhanced protein expression. The results showed that the nanoparticles were superior at inducing high levels of systemic antibody responses compared with naked DNA when delivered by the intradermal route in BALB/c mice. In addition, the nanoparticles induced higher levels of IgM, IgG and IgA antibodies.

Cui *et al.* [143] used PLA nanoparticles as a carrier for HIV-1 Tat (1 – 72). The authors related that up to now no studies (*in vitro* and *in vivo*) have verified whether Tat (1 – 72) presents an immunosuppressive effect because several studies demonstrated that Tat (1 – 86) is immunosuppressive. The results confirmed that Tat (1 – 72) is immunogenic and non-immunosuppressive. Immunization of BALB/c mice with Tat-coated nanoparticles resulted in antibody levels (IgG and IgM) comparable to those elicited from Tat and the aluminum adjuvant. Further investigation is required to verify whether these nanoparticles are able to enhance both antibody and cellular immune responses.

Ataman-Önal *et al.* [144] obtained surfactant-free PLA nanoparticles with a largely negative surface charge, allowing adsorption of the HIV-1 p24 capsid protein. The antigenicity and immunogenicity of proteins on these nanoparticles were well preserved. These nanoparticles induced potent antibody

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responses, including strong CTL responses in mice as well as $CD4^+$ and $CD8^+$ IFN-γ-producing T-cell responses in macaques. This new protein delivery system confirmed the promising potential of charged particle vaccine development. In another study [145], this group investigated whether the powerful carrier system could be used for the delivery of a divalent candidate vaccine against HIV-1. As *gag–env* combinations are widely tested in HIV vaccine research, the authors added the gp120 envelope glycoprotein to the PLA-p24 formulation. The results showed that two different antigens can be adsorbed together onto particulate carriers and that the mass ratio of proteins is identical in the feed and on the particle surface. Furthermore, the structural integrity of p24 and gp120 proteins was extremely well maintained following adsorption, as assessed by antibody-specific and soluble CD4 (sCD4) binding assays. Finally, the divalent PLA-p24/gp120 formulation was highly immunogenic for both antigens, which was indicated by the induction of antigen-specific antibodies in the mice.

Guillon *et al.* [146] quantitatively and qualitatively compared the effect of different adjuvants (PLA nanoparticles versus an emulsion) in the induction of antibody responses using three HIV-1 antigens: p24gag, wild-type Tat and a mutated, detoxified form of Tat. The main result was that the nature of the adjuvant had consequences on the spectrum of specificity induced, depending on the antigen: the PLA adjuvant induced an anti-p24 response that was more focused on an immunodominant domain compared with the response with the emulsion. With wild-type Tat, no difference was observed between adjuvants in the spectrum of the induced immune response specificity. By contrast, detoxified Tat coated on PLA increased the number of epitopes recognized by serum IgG compared with those as a result of the emulsion adjuvant. These results emphasized that the comparison of adjuvants required assessment for each candidate vaccine antigen rather than for a model antigen because the qualitative differences in outcome vary among antigens.

Castaldello *et al.* [147] developed PMMA-PEG nanoparticles by emulsion polymerization and characterized them *in vitro* and *in vivo* for DNA vaccine applications. The nanoparticles reversibly adsorbed large amounts of DNA, mainly through electrostatic interactions, preserved its functional structure, efficiently delivered DNA intracellularly, and were not toxic *in vitro* or in mice. Furthermore, two intramuscular immunizations (4 weeks apart) with a very low dose (1 μg) of the plasmid pCV-Tat delivered by these nanoparticles, followed by one or two protein boosts, induced significant antigen-specific humoral and cellular responses and greatly increased the Th1-type T-cell responses and CTLs against HIV-1 Tat.

Wang *et al.* [148] suggested that nanoparticles can be used as an efficient antigen delivery system to DCs for a variety of vaccines, such as an anti-HIV-1 vaccine. Dendritic cells play a major role in activating and shaping the adaptive immune response. Indeed, the loading of dendritic cells with a particulate antigen adsorbed onto polymeric nanoparticles can significantly increase the potency of both cellular and humoral immune responses after immunization. The authors constructed an efficient protein-based vaccine using biodegradable poly(γ-glutamic acid) (γ-PGA) nanoparticles, which were capable of inducing potent cellular immunity. A significant expansion of $CD8^+$ T cells specific to the major histocompatibility complex class I-restricted gp120 epitope was observed in mice intranasally immunized once with gp120-carrying nanoparticles, but not with gp120 alone or gp120 together with the B-subunit of cholera toxin. Long-lived memory $CD8^+$ T cells were also elicited. Also, the authors compared γ-PGA nanoparticles with polystyrene nanoparticles and found that the former were much stronger inducers of antigen-specific $CD8^+$ T-cell responses than non-biodegradable polystyrene nanoparticles [149]. In some other work from this group, nanoparticles play a critical role in inducing cellular immune responses. The uptake of ovalbumin by DCs was markedly enhanced by γ-PGA nanoparticles, and the ovalbumin was gradually released from nanoparticles into the cells. In addition, γ-PGA nanoparticles appeared to have great potential as an adjuvant because they induced DC maturation. Although DC maturation was induced not only by ovalbumin-encapsulating nanoparticles but also by a simple mixture of ovalbumin and nanoparticles, only the DCs exposed to ovalbumin-nanoparticles could strongly activate antigen-specific IFN-γ-producing T cells. Subcutaneous immunization of mice with HIV-1 p24-encapsulating nanoparticles activated more antigen-specific IFN-γ-producing T cells in the spleen and induced p24-specific serum antibodies when compared with immunization with p24 alone. Like ovalbumin, the mixture of p24 and nanoparticles also induced antigen-specific serum antibodies but did not activate IFN-γ-producing T cells in spleen cells. The authors concluded that gamma-PGA nanoparticles encapsulating various antigens may have great potential as new and efficient protein-based vaccines against infectious diseases, including HIV-1 infection [150]. Recent results published by the group showed that γ-PGA nanoparticles carrying gp-120 induced stronger gp120-specific cellular and humoral immunity than vaccination with gp120 alone in rhesus macaques, but there was no protective effect against inoculation of simian and human immunodeficiency chimeric viruses [151]. Aline *et al.* [152] investigated the feasibility of DC uptake of p24 on the surface of surfactant-free PLA nanoparticles. After immunization, the p24-coated nanoparticles were efficiently taken up by mouse DCs, which then underwent maturation. The nanosystem also induced serum and mucosal antibody production and elicited strong systemic and local lymphoproliferative responses that were correlated with a Th1/Th2-type response and systemic CTL responses in the immunized mice. Thus, the DCs pulsed with antigen-loaded PLA nanoparticles may provide a new delivery tool for cellular vaccination against chronic infectious diseases.

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4. Conclusions

This review has explored the application of polymeric nanoparticles as carriers for ARV therapy for AIDS treatment and as adjuvants for anti-HIV vaccines. Various nanostructures have shown the ability to improve the efficacy of several ARV treatments while reducing their toxicity. These systems have been shown to provide higher and sustained drug levels in known HIV reservoir sites. The development of nanotechnology-based drug delivery systems is a promising strategy to improve the efficacy and safety of current ARV therapy. The use of nanoparticles as adjuvants for vaccines has produced interesting results, which contribute to the efforts in the development of an efficient and safe anti-HIV vaccine formulation.

5. Expert opinion

It has been almost three decades since the discovery of HIV as the cause of AIDS, and the growing number of HIV-infected individuals and AIDS-related deaths reflects the efforts required to control this infectious disease. The therapeutic options for HIV infection continue to expand. New targets, such as viral entry and integration, have recently been exploited successfully. However, HIV-infected patients in need of treatment are at present committed to lifelong suppressive therapy, and non-compliance leads to a rapid increase in viral load. Despite significant advances that have been made in understanding the mechanism of HIV infection and in identifying effective treatment approaches, optimal treatment strategies for AIDS remain a major challenge.

A major obstacle in HIV eradication is the ability of the virus to remain latent in cellular (macrophages, $CD4^+$ T cells and DCs) and anatomical reservoirs (CNS, lymphoid organs and male urogenital tract). Latently infected cells can escape the viral immune response and persist for long periods of time despite the presence of successful HAART.

So far, there are > 25 drugs approved for AIDS treatment. HAART comprises the combined use of three or more anti-HIV drugs, with the objective of reducing the individual drug dose levels, reducing the risk of drug resistance and acting on the different stages of viral replication, which increases the efficacy of treatment. The effect of ARV therapy on the quality of life of HIV patients has been enormously positive; however, toxicity, adverse drug reactions, suboptimal bioavailability owing to poor physicochemical properties, impaired biodistribution in HIV reservoirs, emergence of drug resistance, requirement of drug monitoring and lifelong adherence are the problems associated with ARV treatment. ARV drugs used in current AIDS therapy have some pharmacokinetic limitations. Most ARV therapies available are formulated as solid dosage forms for oral administration. The drugs delivered by this route suffer significant first-pass effect and variation in absorption, and they are also degraded in the gastrointestinal tract by enzymes and pH conditions. Thus, the drugs have short mean half-life, and the duration of drug action is limited, which necessitates therapy with the drug in large and frequent doses, resulting in a rise of toxic effects. The use of new drug delivery systems for oral delivery of ARV therapy is an alternative to circumvent these problems. Also, to reach the therapeutic target sites, ARV drugs must cross several biological barriers, such as mucous membranes, the BBB and cell membranes. The physicochemical and metabolic properties of these drugs contribute to reducing the amount of drug in the blood and affected tissues. Variability also occurs in the bioavailability profile of ARV drugs and represents a significant factor in the failure of some drug regimens. The development of nanotechnology-based delivery systems for ARV drugs has focused on overcoming these barriers. Nanotechnology-related rational targeting may improve the therapeutic success by decreasing adverse drug effects and requiring less frequent administration regimens, resulting in patient compliance and higher adherence.

The great goal to improve the therapeutic effect of ARV drugs requires overcoming the pharmacokinetic limitations (rapid hepatic metabolism, low bioavailability and short half-life), viral resistance and cellular and anatomic barriers. Nanotechnology can solve problems of drug formulation (poor water solubility and lack of stability) and pharmacokinetic drawbacks, such as bioavailability, short half-life and biodistribution (viral targeting tissues), that are associated with current HAART. The nanometric size of these carrier systems allows efficient crossing of biological barriers, amelioration of tissue tolerance, and improved cellular uptake and transport, and thus, the carrier size enables efficient delivery of the therapeutic agents to the target sites. The nanosystems can improve therapy by controlling drug concentrations in target cells. The literature describes a wide range of nanoparticulate carriers intended for AIDS treatment. Some studies have described ARV drugs that are successfully delivered or targeted using different nanosystems. Polymeric systems include nanoparticles, dendrimers, micelles and nanoconjugates; non-polymeric devices include liposomes, nanoemulsions and solid lipid nanoparticles. The polymeric nanoparticles are attractive carriers because they present higher drug loading capacity and the potential to target HIV reservoirs by manipulation of their surface characteristics. These nanoscale systems have been used successfully in other diseases such as cancer, and therefore there is a better understanding of the practicalities and technicalities associated with their clinical development.

An issue related to the development of new drug delivery systems for ARV therapy is that AIDS treatment involves combination drug therapy. Few studies have developed polymeric nanoparticles for the simultaneous loading of two or more ARV drugs. This issue must be explored and considered thoroughly in the design of nanostructures for ARV delivery and targeting. Another area lacking research is the delivery

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of ARV drug-loaded polymeric nanoparticles by the intranasal route to the brain. This direct nose-to-brain drug delivery route represents an interesting strategy to deliver ARV therapy to the brain. The applicability and development of polymeric nanoparticles for this brain-delivery route is a promising area of research. Finally, it was observed in this review that most studies involving ARV-loaded nanoparticles are based on *ex vivo* or *in vivo* studies using rats. These data show that the research in this field is at an early stage.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

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