Volume 4, Issue 4, 2014, 820-831 **ISSN 2069-5837**

Biointerface Research in Applied Chemistry

www.BiointerfaceResearch.com

Review Article **Open Access Journal**

Received: 22.06.2014 / Revised: 30.07.2014 / Accepted: 08/.08.2014/ Published on-line: 15.08.2014

Nanotechnological interventions in HIV drug delivery and therapeutics

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ABSTRACT

Nanotechnology has shown tremendous applications in healthcare promising better alternatives to diagnose and treat HIV-1 infection. The significant drug load, lengthy regimen, emergence of drug resistance and poor patient compliance result in the poor management of HIV-1 patients. Attributes like sustained release, increased half life, higher drug concentrations at target sites, reduced toxicity and lesser side effects have made nanotechnology based therapy a desirable prospect. It targets not only active virus but also targets latent HIV in anatomically privileged sites. New strategies like RNAi interference have the added advantage of specific targeting and targeting persistent reservoirs. However because of the severity of the disease, there is a strong urge to develop better approaches with some concomitant regimens using nano-particles to diagnose and treat HIV patients. The current review highlights some of the advancements in HIV-1 related nanotherapeutics.

Keywords: *Nanotechnology, HIV, Drug Delivery, Therapeutics.*

1. INTRODUCTION

Human immunodeficiency virus (HIV), the causative agent of acquired immunodeficiency syndrome (AIDS) is a leading cause of morbidity and mortality worldwide. At present more than 35 million people are infected worldwide, especially in countries with low income from Asian and African continents. HIV is a Lentivirus (a member of the retrovirus family) that causes (AIDS). The condition is characterized by severe impairment of immune system resulting in markedly decreased $CD4^+$ T cells. Decreased $CD4^+$ T cell count makes the patient prone to various life-threatening opportunistic infections. HIV entry into the cell occurs via gp120 interaction with surface receptors $CD4^+$ (cluster designation 4) and one of two chemokine co-receptors (CCR5 or CXCR4) [1-2]. The virus then fuses with the host cell releasing its genetic material RNA and various enzymes, i.e. reverse transcriptase, integrase, ribonuclease and protease into the host cell. Reverse transcriptase converts the single-stranded HIV RNA to double-stranded HIV DNA. The newly formed HIV DNA enters the host cell's nucleus, where integrase "hides" the HIV DNA within the host cell's own DNA. The integrated HIV DNA is called provirus which may remain latent for several years, producing few or no new copies of HIV. When the host cell receives a signal to become active, the provirus use host`s RNA polymerase to transcribe HIV DNA into mRNA, which is then translated into specific proteins. An HIV enzyme called protease cuts the long chains of HIV proteins into smaller individual proteins. These synthesized proteins associate with HIV's RNA a new virus particle is assembled and released after cell lysis. At present there is no cure for HIV and available therapies; highly active antiretroviral therapy (HAART) and cART only suppress viral replication and brings level down to undetectable limit 50mg/l with virus still persisting in sanctuary sites, such as the liver, brain, gut, kidney, testes and secondary lymphoid tissue [3-4]. Further extremely lengthy regimen of HAART and cART therapies are related to poor patient compliance, drug intolerance, toxicity, viral relapse after some time [5-7] and emergence of drug resistant viral strains exacerbates the situation. Nanotechnology holds a great promise in anti-retroviral drug/gene delivery as it not only improves drug/gene delivery to target tissues but also targets viral sanctuary sites to eliminate latent HIV [7]. With a dearth of novel compounds in the pipeline and lethargic drug discovery, nanotechnology makes the current regimen more efficacious with a considerable improvement in quality of HIV patients. It modulates the delivery of current drugs in an effective manner via various novel drug delivery systems thereby increasing the drug bioavailability and residence time at target sites. Nanotherepeutics therefore offers a promising tool to overcome these limitations and achieve controlled, constant and specific release of desired drugs to the target sites. The pharmacokinetics, *in vitro*, *ex vivo*, and *in vivo* studies of various anti-HIV nanoencapsulated drugs have been reviewed in the manuscript.

2. DIFFERENT TYPES OF NANOPARTICLES FOR HIV

Nanoparticles (NPs) are solid colloidal particles generally in the size range of 10–100 nm [8]. Therapeutic agents either alone or in combination can be entrapped or chemically linked to the surface of these NPs. NPs act as masking agents, protect the encapsulated drugs against enzymatic degradation offering increased stability and half life to encapsulated drugs in biological fluids [8]. The advent of multifunctional NPs has enabled to simultaneously deliver therapeutic agents and also act as imaging agents for real-time tracking within cells [9]. NPs made from natural polymers such as gelatin, albumin offer biocompatibility and biodegradability while inorganic synthetic NPs such as gold, iron oxide offer uniformity in size and stability [9]. Due to their small size they are effectively taken up by cells and maintain a therapeutic concentration at target sites. A wide array of NPs used

in HIV-1 therapeutics and diagnosis have been summarized in table 1.

3. NANOPARTICLES IN DIAGNOSIS OF HIV

Nanotechnology shows significant properties such as nano-size, target specificity and molecular fluorophores which make these NPs as promising bio diagnostic tool. Traditional methods of diagnosis of HIV rely on serological and nucleic acid based techniques. The most commonly methods used for detection of HIV are ELISA, EIA and p24 (quantifies p24 core protein). Traditional techniques have limitation such as no early detection and no accurate viral load estimation, which could be overcome by using nanotechnology in HIV diagnosis. Recent advancement in nanotechnology led to the development of AuNP-based biobarcode amplification assay (AuNP-BCA). AuNP-BCA is a highly sensitive technique capable of detecting HIV-1 p24 antigen at very low concentration level (0.1pg/ml) and offers 100-150 fold improved recognition limit over conventional ELISA method. In the same study AuNPs when replaced with Europium-based NPs in BCA assay raise the efficacy of detection for HIV-1 p24 antigen, simultaneously lowering the incubation time [23]. In a research conducted by Jingfeng et al 2009, they developed a visual DNA microarray simultaneous, sensitive and specific detection of HIV-1 based on gold label silver stain and coupled with multiplex asymmetric polymer chain reaction (PCR). The conventional microarray technique for detection relies on confocal scanner which makes it expensive technique. In a recent technique 5'-amino modified oligonucleotides were immobilized on glass surface acting as capturing probes that bind to the complementary biotinylated target DNA. Au-conjugated streptavidin specifically binds to the biotin molecules in microarray; this gives a black spot in microarray which is the result of the formation of silver precipitate onto AuNPs and bound to streptavidins [24]. Quantum dot based HIV capture and imaging in a microfluidic channel can detect HIV in microliter quantities (10 µl) of blood from infected patient in anti gp 120 antibody immobilized microfluidic chip. In this technique two color quantum dots (Qdots525 and Qdots655 conjugated with streptavidin) were used to identify captured HIV by simultaneous labeling the envelop gp 120 and high mannose glycans [25]. In a recent study different unique affinity nanotraps were used to capture HIV-1 virions in culture supernatant and tat/nef proteins spiked in culture medium. Nanotrap particles NT082, NT084, NT080, and NT086 captured tat, tat peptide, nef protein and membrane associated nef respectively at a high level and measured by western blotting. Another nanotrap particle NT086 conjugated with bait Methacrylate could successfully capture high amount of whole HIV-1 particle and gp41 and measured by reverse transcriptase assay. Demonstration of captured infectious HIV-1 by nanoparticles is given by functional trans-activation in TZM-bl cells [26]. HIV-1 p24 antigen is a major viral component and plays an important role in the early stage of infection and transmission of HIV-1 from infected mother to infants. A nanoparticle based bio-barcode amplification assay was used to detect HIV-1 p24 antigen and quantitative measurement of captured p24 antigen done by PCR and gel electrophoresis. Microplate wells or magnetic microparticles (MMPs) were coated with G12 mAb to capture free p24 antigen. The complex 1G12 with p24 antigen captured 1D4 mAb coated gold nanoparticle probes (GNPs) containing double-stranded DNA oligonucleotides in a sandwich format. One of the free strands of oligonucleotide released upon heating was amplified by PCR and quantified while other strand remains covalently immobilized [27]. During initial infection anti-HIV antibodies are produced after three months of infection thus detection during this period is challenging. A US patent US20140045169 A1 provides a novel method for detecting HIV at the early stages of infection particularly from saliva samples, by using glycan immobilized metal NPs. Glycan covers most of our cell surfaces and is contacted by HIV-1 during infection. The glycan NPs when contacted with saliva sample specifically bind HIV-1, the mixture is then concentrated and HIV-1 determined by appropriate detection methods [28].

4. NANO-ENCAPSULATED ANTI-HIV DRUGS

4.1. Microbicides

Dendrimers are potential microbicidal agents, known to inhibit HIV-1 infection [29-36]. SPL7013 gel (vivagel) is a dendrimer based microbicide used for prevention against HIV and HSV (herpes simplex virus) infections [29]. The dendrimer inhibits HIV infection not only by preventing viral entry but also by inhibiting HIV-reverse transcription in HIV-infected cells [37]. Formulated SPL7013 showed prolonged *in vivo* protection against HSV-2 in mouse vaginal transmission model [38]. SPL7013 inhibited viral entry in both CXCR4-(X4) and CCR5 using R5 HIV-1 strains [39]. A single vaginal administration of SPL7013 in women resulted in >90% HIV-1 and HSV-2 inhibition after 24 hrs in 6 out of 11 women. The microbicide was quite safe showing no symptom of vaginal, vulvular or cervical irritation. RANATES (Regulated upon activation, Normal T Expressed and Secreted) a naturally occurring chemokine binds specifically to T-lymphocytes and monocytes have been developed as microbicide against HIV. Ranates specifically inhibit R-5 tropic HIV-1 by blocking virus binding to CCR5. PSC-RANATES an amino terminus modified analogue of chemokine ranates has more potent anti-viral activity acts via blocking CCR5 expression. It shows of high level in vitro HIV-protection and in vivo activity against SHIV in rhesus macaque model [40]. PSC-RANATES when encapsulated in PLGA nanoparticles showed enhanced mucosal tissue penetration providing sustained control drug release for the prevention of HIV-infection [41]. Crespo et al prepared a combination of anionic carbodilane dendrimers and ARVs for inhibition of HIV-1. Anionic carbisialne dendrimers G2-STE16, G2-S24P and G2-S16 inhibit viral entry by targeting CD4+/gp120 and CCR5 or CXCR4/gp120 interaction (Figure 1). These dendrimers, when complexed with ARVs, such as Tenofovir (TFV) or Maraviroc (MRV) act as potent microbicides by inhibiting viral entry and transcriptase reversing steps of HIV infection [42]. G2-STE16 was the most potent dendrimer which

when complexed with TFV or MRV showed significant anti-HIV activity. G2-STE16/TFV and G2-STE16/MRV displayed synergistic inhibition against R5- HIV-1, X4-HIV-1 and X4/R5-HIV-1 strains ranging from 75% to 100%.

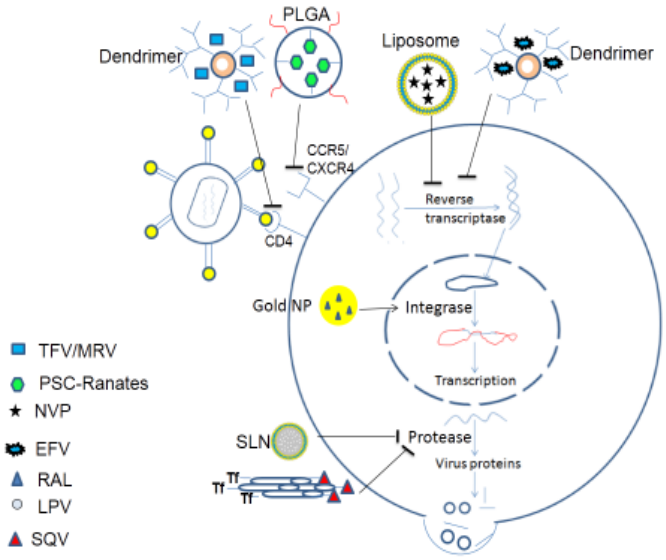

Figure 1. Schematic representation of the influence of some of the nanoencapsulated drugs on HIV-1 replication.

4.2. Non nucleoside reverese transcriptase inhibitors (NNRTI)

Rilparavine a second generation NNRTI with a half-life of 38 hr was approved by FDI in 2011 for use in U.S. Beart et al prepared a nanoformulation of the Rilparavine stabilized by polyethylene-polypropylene glycol (poloxamer 338) and PEGylated tocopheryl succinate ester (TPGS 1000) and studied its pharmacokinetics in mice and dog. The Rilparavine formulation administered as single intramuscular or subcutaneous injection in mice and dogs resulted in sustained release over 3 months in dogs and 3 weeks in mice. Plasma pharmacokinetics, injection site concentrations, disposition to lymphoid tissues and tolerability were evaluated for its use as once-monthly drug in humans. Thereby the potential of this long acting drug formulation could improve compliance in HIV patients and prophylaxis against HIV transmission. [43-44]. Efavirenz (EFV) is an oral NNRTI class of retroviral. However its high lipophilicity and consequently poor aqueous solubility results in low bio absorption and bioavailability [45]. It also imparts a strong and prolonged burning sensation to the mouth when taken empty stomach in order to minimize its neurological and psychiatric effects [46]. Preparation of EFV nanoformulation, therefore provides an alternate approach to achieve desirable attributes, along with reduced side effects. Dutta et al prepared EFV loaded tufstin conjugated 5th generation poly (propyleneimine) dendrimers (TuPPI). Tufstin is a tetrapeptide produced by enzymatic degradation of immunoglobulin G, binding specifically to mononuclear phagocytic cells and enhances their natural killer activity [47]. TuPPI prolonged the in vitro release of EFV up to 144 hr against 24hr of PPI. TuPPI loaded EFV also showed a 34.5 times higher cellular uptake and reduced viral load by 99% at a concentration of 0.625 ng/ml which was more significant in HIV infected macrophages than uninfected cells. Enhanced cellular uptake, reduced toxicity and inherent anti-HIV activity were key attributes of TuPPI making them promising anti-HIV candidates [48]. In the same study they also prepared t-Boc-glycine conjugated PPI dendrimer (TPPI) and mannose conjugated dendrimer. Mannose conjugated dendrimer showed an appreciable increase in cellular uptake of EFV by Monocyte/Macrophage cells which was 12 times higher than free drug and 5.5 times higher than that of (TPPI) [49]. Madhusudan et al in a study designed and evaluated different parameters of EFV loaded SLN. Prepared SLN were in range of 80-100nm, with a sustained release, decrease dosing and fewer side effects. [50]. In another study EFV NPs using methacrylate polymers were synthesized and *in vitro* evaluations such as particle size, morphology, drug release, solubility changes, biocompatibility and cytotoxicity were assessed. EFV-NPs showed better uptake of NP than free drug in monocytes/ macrophage [51]. Ramana et al developed nepiravine (NVP) loaded liposomal formulation and studied its different parameters and concluded that the formulation has an efficient targeted delivery of the anti-retrovirals to the selected compartments and cells with reduced systemic toxic side effects [52]. As discussed elsewhere in the paper transferrin receptors help in transport of selected bio molecules across the BBB. Kuo et al prepares poly (lactide-co-glycoside PLGA nanoparticle grafted with transferrin for enhanced transport across Human Brain Microvascular Endothelial Cells (HBMEC). The Tf/NVP-PLGA NPs were efficient carriers in targeting delivery across HBMECs for viral therapy [53]. Sheqokar prepared NVP nanosuspension which had enhanced cellular uptake and prolonged residence in lymphatic circulation. High MRT values confirmed enhanced bioavailability and prolonged residence of drug at the target site [54]. In a separate work they developed nevirapine nanosuspension with surface modification via albumin, polysaccharite and PEG to enhance its targeting potential. Surface coated nanosuspension when administered intravenously in rats showed enhanced bioavailability, antiretroviral drug accumulation and prolonged residence in organs such as brain, liver and spleen compared to free drug [55]. Squalene a natural terpenoid when conjugated with nucleoside analogs produces amphiphilic prodrugs capable of self assembling in water as nanoassemblies of 300nm size [56]. The process is called as saquenoylation which allows synergistic effects of both the prodrug and nanocarrier. Hiliareu et al showed that saquenoylated NNRTI such as dideoxycytodine (ddc) and didanosine (ddi) when incubated in HIV infected PBMCs enhanced the antiviral delivery of the parent drug. Sq-NNRTI orally administered in rats exhibited increased levels in plasma and target tissues. Sq- NNRTIs nanoassemblies not only enhanced cellular uptake and improved bioavailability but also significantly increased anti-HIV efficacy [57].

4.3. Nucleoside reverse transcriptase inhibitors (NRTI)

Lamivudine (LAM) is a NRTI class of drug with a short half life of 5-7 hr. Wang et al prepared lamivudine loaded PLGA NPs coated with BSA. The LAM-PLGA-BSA (LPB) NPs were internalized into human liver cells with in a short time and increased gradually with prolongation of incubation. After ingestion the LPB NPs could not enter cell nucleus but reside with in lysosome or transfer to cytoplasm [58]. Tamirhrashi et al

prepared polymethyl acrylic acid nanoparticle loaded with lamivudine. The nanoparticle showed a slow and constant release of LAM with constant drug plasma concentration increasing therapeutic efficacy. The polymethyl acrylic acid nanoparticle overcome and alleviates the drawbacks of LAM [59]. LAM loaded chitosan nanoparticle prepared by ionic gelation of chitosan with tripolyphosphate anion (TPP). The developed nanoformulation showed sustained release over 24 hrs which was efficient than lamivudine conventional dosage forms [60]. Surfactant coated (Tween 80) and uncoated chitosan nanoparticle containing LAM were prepared for targeted delivery to the brain. Drug loaded nanoparticle showed good stability for 60 days. Tween 80 coated nanospheres were efficient and cheaper carrier for targeted delivery of LAM to the brain against HIV associated CNS disorder [61].

Zidovirudene (AZT) is a potential drug used in combination with other anti-retroviral drugs. Due to extensive first by pass metabolism AZT has low oral bioavailability and has half life of 1 hr. Carvalho et al showed that systems formed in PPG-5-CETETH-20/oleic acid/water composition have controlled AZT release over a longer duration and improved AZT incorporation into the target tissues [62]. Rubiana et al encapsulated AZT on biodegradable poly(L-lactide) (PLA) or poly(L-lactide)-poly(ethylene glycol) (PLA-PEG)-blend NPs by the double-emulsion solvent-evaporation method. The study showed that PEG presence influenced all of the analyzed physicochemical parameters and increased drug released with the PEG presence in the blend, thereby more promising carriers for AZT [63]. Rubiana et al in a similar study showed intranasal delivery as an effective route for administration of AZT by PLA and PLA-PEG blend NPs. They showed that PLA and PLA-PEG blend NPs had same morphology but particle size and zeta potential were changed by PEG. PLA-PEG blend nanoparticle showed greater efficiency with T(max) twice of PLA nanoparticle. The relative bioavailability of AZT-loaded PLA-PEG nanoparticle was 2.7 times more relative to AZT-loaded PLA nanoparticle and 1.3 times more relative to aqueous solution formulation, thereby depicting PLA-PEG blend NPs as potential carrier of drug via intranasal route [64]. However in vivo and animal model studies of these NPs are lacking to authenticate their anti-HIV efficacy.

Stavudine is a water soluble drug with a serum half life of 1 hr. WHO in 2009 phase out the use of Stavudine, on account of its long term irreversible side effects such as peripheral neuropathy, lipodystrophy, lactic acidosis etc. Thus a new formulation with increased cellular uptake,sustained release and lesser side effects could reestablish its use as first line ARV drug. Garg et al reported that stavudine loaded galactosylated liposomes had a reduced hepatic toxicity, enhanced cellular uptake and half-life of stavudine [65]. Garg et al in another study prepared Stavudine-loaded mannosylated liposomes and assessed their *in vitro* anti-HIV-I activity. The mannosylated liposomes were more potent in inhibiting HIV infection compared to free drug and uncoated liposomes. The mannosylated liposomes showed 14-20 and 1.4-2.3 times lower p24 levels than free drug and uncoated liposomes respectively [66]. In another study Sheqokar et al developed stavudine solid lipid nanoparticles. *Ex vivo* cellular uptake studies of stavudine loaded NPs in macrophages showed enhanced uptake of these NPs compared to pure drug solution. The lymphatic drug levels and organ distribution studies depicted that prepared SLNs has prolonged residence in splenic tissues [67].

4.4. Protease inhibitors

Saquanavir (SQV) is a water soluble drug with low oral bioavailability. Mahajan et al developed a nanoformulation *via* incorporating SQV within Tf-conjugated quantum rods. The QR-Tf-SQV nanoformulation was checked for ability to cross the blood brain barrier (BBB) and antiviral efficacy against HIV- 1 infected peripheral blood mononuclear cells (PBMCs). The BBB crossing ability of these NPs was significantly increased with brain microvascular endothelial cells (BMVECs), showing a significant uptake of QR-Tf-SQV nanoparticles. The nanoparticles also brought an appreciable decrease in HIV-1 viral replication in the PBMCs [68]. Recently Ramana et al used chitosan based nano-formulation for the delivery of SQV. These chitosan loaded NPs showed increased drug loading efficiency ~75% and increased cell targeting efficiency of ~95% which was about 2.5 timed more than free drug. These NPs when also evaluated for anti HIV efficacy against NL4-3, Indie-C1 HIV, Jurkat and CEM-CCR5 cell lines showed increased anti-HIV potential. Chitosan-NPs thus were potent carriers for SQV increasing its cellular uptake, targeting efficacy and anti-HIV efficacy [69].

Dou et al formulated a nanoformulation of the drug indanavir with lipoid E80 for effective delivery to various tissues. The indanavir nanosuspension was then loaded into macrophages and these loaded macrophages when injected intravenously into mice, resulted in increased distribution in the lungs, liver and spleen. Rodent mouse model of HIV brain infection when intravenously administered with a single dose of nanoparticle loaded macrophage showed significant antiviral activity in brain [70].

Lopinavir (LPV) a less soluble drug with a low bioavailability due to high first pass metabolism, creating need for encapsulation of the drug for efficient and specific release of the drug into target tissues [71]. LPV loaded glycerol base solid lipid nanoparticles were developed to target intestinal lymphatic vessel. Study showed that bioavailability of the drug was significantly enhanced with increased cumulative percentage dose of LPV secreted into lymph [72]. Rahul et al prepared polycarpolactone based NP of LPV and studied its various parameters. The *in vivo* performance of the nanoformulation was assessed in rats. At the end of 8h less than 20% cumulative release was observed. Lower release ensured high stability and increased probability of NP taken by lymphatic system. The prepared nanoformulation was stable and showed extended release, increased oral bioavailability and reduced systemic clearance [73].

4.5. Integrase inhibitors

Most of the anti-HIV drugs are unable to cross the BBB. Garrido et al assessed the role of gold NPs (GNPs) with an aim for improved drug delivery to HIV reservoirs in the brain. Gold nano particle not only effectively enter into lymphocytes, macrophages, astrocytes and HBMECs but able to cross BBB both *in vitro* and *in vivo*. They also assessed the anti-HIV efficacy of drug raltegravir. Reltagravir derivative when conjugated to GNPs and

tested for anti-HIV activity in primary PBMCs resulted in 4 fold reduced HIV replication after 5 days of incubation [74].

5. ANTI-HIV NANO-VACCINE AND NANO-THERAPEUTICS

5.1. Nanovaccines

Dermavir is a topical therapeutic vaccine comprising a single plasmid DNA expressing 15 HIV antigens, a synthetic pDNA nanomedicine formulation and a dendritic cell-tar getting topical vaccine administration (Dermaprep). Dermavir's novel mechanism of action involves activation of epidermal Langerhans cells which capture dermavir nanopaticles and migrate to local lymph nodes. Inside lymph nodes these cells mature into dendritic cells which present dermavir encoded epitopes to naïve T cells. HIV specific CD4+ and CD8+ precursor T-cells then proliferate and destroy HIV-infected cells throughout the body [86]. Toke et al in a study developed a formulation in which pDNA was complexed with polyethyleneimine (PEI) that is mannobiosylated to target antigen-presenting cells to induce immune responses. Their results showed that the new formulation was capable of maintaining biological activity and physical stability of nanomedicine [87]. Kolonics in another study developed alexa546-dermavir nanoparticles by covalently bounding Alexa546 succinimidyl ester (Invitrogen) to the amine of PEIm. They showed that when dermavir topically administered to the mouse ear penetrates through murine epidermis and concentrates around LCs in the epidermis. After 1 hr of treatment Alexa546-Dermavir was endocytosed and after 9 hrs the fluorescence of Dermavir was observed intracellularly close to the nucleus in LCs proving its successful uptake. However there was a decrease in Alexa546-DV fluorescence after 9 and 24 hours suggesting its degradation and release of plasmid DNA into Langerhans cells [88].

An immunogen gp41-54Q-GHC based on the membrane proximal external region of HIV-1 glycoprotein (gp41) was used to design a biodegradable nanoparticle-based nanovaccine. Polyanhydride polymers 1,6-bis(*p*-carboxyphenoxy) hexane (CPH) and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) were used for the synthesis of nanoparticles which maintain desirable characteristics as adjuvants, immune modulators, activation of antigen presenting cells, induction of high titer delivery vehicles. NPs encapsulating gp41-54Q-GHC were capable of stimulating a potent immune responses when injected subcutaneously in BALB/C mice [89].

Qiao et al evaluated the potential of pegylated poly (2-(dimethylamino) ethyl methacrylate) (PDMAEMA) NPs as a gene delivery vector. Peg-PDMEAMA complexed with HIV gag gene upon intranasal administration in mice efficiently condensed DNA and elicited a stronger immune response compared to naked plasmid DNA. Further PEGylated PDMAEMA were also able to induce cytokines production by murine macrophages suggesting the potential of PEGylated PDMAEMA NPs simultaneously acting as DNA delivery vectors and adjuvants enhancing the immunogenicity of DNA vaccine [90]. Altman et al prepared PLA NPs coated with HIV-p24 protein for inducing immune responses in mice, rabbit and macques. The antigen loaded NPs upon subcutaneous administration into animal models not only elicited increased antibody titers but also strong CTL responses. Further p24-PLA NPs were also induced TH1-biased cytokine release by increasing IFN-γ producing CD4+ and CD8+ T cells [91]. Xu and co-workers used fullerenol capable of self assembling into virus size NPs to induce immune responses comparable to HIV DNA vaccine. Fullerenol acts as dual functional nanoadjuvant significantly enhancing the immune response to HIV vaccine probably via activation of Toll like receptors signaling pathways [92]. Tian et al fabricated a peptide based nano-fibrous hydrogel that act as a nanovector for the delivery of HIV vaccine [93]. The nanovector was quite safe and able to condense DNA hence eliciting a potent immune response against HIV. γ-PGA is a naturally occurring water-soluble, biodegradable, edible and non-toxic poly(amino acid)s form 200nm NPs in water. γ-PGA NPs are known for better uptake and adjuvant properties for DC cell maturation [94-95]. γ-PGA when complexed with HIV-1 gp120 or p24 have been found to induce antigen specific cellular immunity in mice [96-97]. γ-PGA NPs carrying HIV-1 gp 120 when injected into macaques induced a strong CD4 T cell immune response against env-gp 120.

5.2. Peptides

Melittin a principal active component of apitoxin (bee venom) was found to be effective against HIV infected cells but is also toxic to normal cells. NPs encapsulating melittin could be novel candidates targeting HIV-1 infected cells with least toxicity to normal cells. NPs loaded with cytolytic melittin peptides could inhibit HIV-1 infectivity by CXCR4 and CCR5 tropic HIV-1 strains. The mechanism behind this killing was that the NPs fusion with the outer envelope of the HIV-virus poking holes in the protective envelope of virus. These initial evidences of nanoparticle mediated HIV inhibition could conceptualize further development of potential microbicides or nano-vaccines against HIV infection [98]. Curcumin, a polyphenol obtained from *Curcumin longa* has excellent antioxidant, anti-inflammatory, anti-microbial and anti-carcinogenic potential. It is also hepato and nephroprophylactic, suppress thrombosis, protects against damage due to myocardial infarction and anti-rheumatic activities [99-102]. Its use has been limited on account of limited bioavailability, poor bio absorption, higher metabolism rate and poor bioavailability. It has been previously reported to inhibit HIV activation and replication [103-104]. However an effective clinical use could not be attributed due to sub therapeutic dosage regime. Gandapu et al described that curcumin loaded apotransferrin NPs capable of increasing the cellular uptake of curcumin via targeting endocytosis promoting cellular receptor. These NPs maintained an effective therapeutic concentration of curcumin inside target cells inhibiting HIV-1 replication simultaneously reducing cytotoxicity.

5.3. Gene Therapy

Gene therapy implies the delivery of RNA or DNA based therapy for the treatment of diseases. Gene therapy particularly RNA-based therapies employing RNAi with extraordinary sequence specificity are an attractive prospect for the treatment, of

HIV [105-108]. However owing to their limitations such as short half-life, poor cellular uptake, low bioavailability sub cellular compartmentalization, safety concerns, unwanted interactions with plasma proteins and the immune system, [109-119] limits their use. A number of nanodrug delivery systems have been developed for the delivery of therapeutic RNA and DNA.

5.3.1. RNAi approach

Most strains of HIV use chemokine receptor CCR5 for entry into target cell. RNAi mediated silencing of CCR5 is an important target in HIV therapy. Liposomal NPs were conjugated with lymphocyte function–associated antigen-1 (LFA-1) for delivering anti-CCR5 to LFA-1 expressing human leukocytes. Upon administration these NPs were selectively taken by T cells and macrophages. These antiCCR5siRNA/LFA-1 I-NPs exhibited leukocyte specific silencing. Humanized mice model challenged with HIV when pretreated with anti-CCR5siRNA showed an increased resistance to infection [120]. Nanoplexes with a diameter around 20mm have also been used for siRNA delivery targeting CCR5gene. CCR5-siRNA nanocapsules when delivered to 293T cells significantly down regulated CCR5 expression. CCR5-siRNA delivered via nanocapsules were more potent in knocking down CCR5-mRNA expression which was <15% compared to lipofectime delivered siRNA which only knock down expression upto 55% [121]. Another siRNA S510, belong to class of mRNA, targeting TAR/polyA region of HIV-1 LTR. S510 siRNA was conjugated with quantum rods (QR) for the delivery of anti-TAR siRNA. An HIV-1 infected THP-1 cell line when treated with QR-S510 siRNA nanoplex resulted in >90% suppression of viral replication after 48 hrs and continued upto1 week post transfection. These NPs exhibited a sustained release of siRNA and were quite effective in delivering siRNA to cells compared to lipofectime. QR-S510 siRNA nanoplex exhibited decreased TAR/polyA gene expression resulting in decreased HIV-1 viral replication as evident by lowered p24 levels [122]. Waber et al synthesized carbosilane dendrimer for the delivery of siRNA to HIV infected lymphocytes. Carbosilane dendrimers (CBS) stably bind oligo and siRNA and bring a sustained release of loaded molecules over longer duration. CBS/siRNA dendriplexes when delivered to PBMC and SUPT1cell line silenced GADPH expression in these cell lines. Inhibition of GADPH expression resulted in inhibition of HIV replication [123]. In a similar study Jiang et al prepared 2G-NN16 carbosilane dendrimers to target HIV infected astrocytes in brain. 2G-NN16 dendrimers loaded with siRNA against GADPH effectively crossed Blood Brain Barrier and liberated siRNA between 12-24 hrs. siRNA/2G-NN16 dendriplexes inhibited the replication of X4-HIV NL4-3 and R5-HIV BaL HIV-1 strains in human astrocytes by down regulating the expression of the GADPH inhibiting HIV-1 replication [124]. Another class of dendrimers G4 (NH+Et2Cl-)96, phosphorus containing cationic dendrimers were prepared for the delivery of siRNA against HIV-Nef gene. G4 (NH+Et2Cl-)96/siNEF dendriplexes when injected into PBMCs resulted in silencing of Nef gene significantly reducing viral replication [125]. Zhou etal employed cationic polyamidoamine (PAMAM) for siRNA delivery to HIV infected mice. A combination of 3 siRNA one targeting HIV-1 tat/rev and two host targets CD4 and TNPO3 were used for suppressing HIV-1 infection. TNPO3 (Transportin-3) and CD4 are host dependency factors essential for establishment of HIV-1 infection. PAMAM dendrimers loaded with siRNA against HIV-1 tat and host CD4/TNPO3 when injected intravenously in a Rag2-/. (Rag-hu) humanized mouse model for HIV infection considerably suppressed HIV-1 infection. These NPs significantly accumulated in PBMCS and liver and protected against viral induced CD4+ T cell depletion. Inhibition of these targets also decreased viral RNA load and protected CD4+ T cell from HIV-1 mediated depletion [126]. Addictive drugs such as methamphetamine potentiate HIV replication in immunocompetent cells including macrophage, monocyte and PBMCs [127]. Methamphetamine increases the expression of Galectin-1 gene in immunocompetent cells making them more susceptible to HIV-1 infection. Galectin-1 a member of B-glactosidase binding lectins that modulate cell to cell and cell to matrix interaction [128]. It participates in cell-HIV-1 interaction and stabilizes this interaction in PBMC and CD4+ T cells [129]. Concomitant incubation of Monocyte Derived macrophages with methamphetamine and galectin-1 enhanced expression of HIV-LTR-R/U5 region and increased HIV-1 p24 antigen production. Gold nanorods complexed with galectin-1/siRNA were used for silencing of galectin-1 gene. GNR/galectin-1/siRNA nanoplex not only silenced galectin-1gene expression but also reduced methamine induced galectin-1 mediated HIV-1 infection signifying the importance of galectin-1 gene silencing in inhibiting HIV-1 replication [130, galectin-1]**.**

5.3.2. Antisense approach

Antisense approach represents a similar approach to that of RNAi targeting HIV-1 mRNA expression. In this approach antisense oligonucleotides complimentary to HIV-1 mRNA inhibit gene expression [131]. A number of approaches have been developed for the delivery of anti sense oligonucleotide into infected cells. However limitations such as poor ability to cross biological membranes, poor bioavailability and rapid biodegradation in biological membranes limit their pharmacological effects. Dianuer et al synthesized antisense oligonucleotides (AS-ODN) and their phosphor analogues (AS-PTO) against HIV-1 Tat mRNA. These oligonucleotides were incorporated into NPs by conjugation with protamine (PM). PM/AS-ODN NPs exhibited increased cellular uptake and sustained release of AS-ODN inside cells while PM/AS-PTO NPs did not release AS-PTO. PM/AS-ODN NPs significantly inhibited tat mRNA HIV transactivation which brought a decreased HIV load. Inhibition by PT-AS-ODN NPs was concentration dependent with maximum reduction at 54% at concentration of 5Um [132]. In another approach HIV antisense oligonucleotides SREV, ANTI TAR and GEM91 were characterized for their interaction with polypropylene dendrimers. GEM91 a 25-mer phosphorothioate oligonucleotide targets HIV gag mRNA, while SREV and ANTI TAR targets rev and tar regions of HIV mRNA respectively. Different generation Poly(propylene imine) dendrimers PPIG2, PPIG3, PPIG4 showed significant interaction with above said oligonucleotides with SREV showing highest and GEM91 least interaction. Further in vivo studies in animal model regarding uptake and anti-HIV efficiency are yet to be done [133].

5.3.3. DNA

Peptide nucleic acids (PNA) are synthetic nucleotides capable of forming triple helix with duplex DNA. PNA have high specificity for DNA and stably bind DNA forming triplex structure inducing DNA repair and producing genome modification [134]. PNA targeted to CCR5 gene in cells naturally mimicked CCR5-Δ32 mutation [135]. CCR5-Δ32 homozygous mutant are almost completely resistant to HIV-1 infection [136-137] while heterozygous individual with single mutant allele also showed considerable reduced progression of HIV-1 [138-139]. PLGA NPs encapsulating PNA and donor DNA were developed for CCR5 gene editing. These NPs effectively entered PBMCs and exhibited highly specific targeting up to 97% of CCR5 gene. Humanized mice challenged with R5 tropic strain of HIV-1 upon treatment with CCR5-NP treated PBMCs significantly reduced plasma viral RNA and levels of CD4+ T cells [140].

5.4. Nano-prophylaxis

Destache and co workers developed a thermosensitive vaginal gel comprising of RAL+EFV loaded PLGA NPs. the gel was quite stable and maintained thermogelation between 30-35°C The gel didn't cause any cytotoxicity in HeLa cells and exhibited a sustained release over time. Transwell experiments of NPs in gel confirmed rapid transfer of fluorescent NPs from the gel and uptake by HeLa cells within 30mins. Although vaginal gel has shown some potential as a prophylactic modality, yet in vivo animal studies are warranted to assess its potential in animal models [141]. A patent no WO 2012068179 A1 claimed a multifunctional biodegradable PEG nanocarrier-based hydrogels for preventing HIV transmission. The hydrogel included a PEG-cross linking unit covalently bound to at least four multi-arm PEG-nanocarrrier. Each of the four nanocarriers comprised different agents which may be either bio adhesion agents, pH-lowering agents, microbicidal-spermicidal agents or agents that inhibit free and cell-associated HIV binding. These agents promote mucosal adhesion, ensure mildly acidic pH, release microbicide and spermicides, and prevent HIV virion binding respectively. The nanocarrier-based vaginal hydrogel thus was proposed to have a role in preventing acquisition and dissemination of HIV through the vaginal mucosa to distant tissues [142]. Another patent entitled Novel DNA-origami nanovaccines with patent no WO 2013119676 A1claimed a composition comprising a DNA-nanostructure complexed to at least one targeting moiety consisting of antigens, aptamers, shRNAs and combinations and methods of use thereof. The nanoformulation could be used in the development of a potential prophylactic nanovaccine [143].

Table 1. Different types of NPs used in HIV therapeutics.

Nanoparticles	Definition	Size(nm)	Characteristics	Ref.
Liposomes	Lipid bilayer like structures encapsulate both hydrophilic and hydrophobic drugs	50-100	Quick clearance from circulation, greater entrapment efficiency, longer half-life in circulation, transdermal delivery	[10]
Dendrimers	Branched macromolecules synthesized via polymerization growing outwards from central core (Generation1-5)	5-20	Polyvalency, i.e. presence of multiple active groups, easy surface modulation	[11]
Carbon nanotubes	Cylindrical Graphite sheets rolled into single or multiwalled tubes	1.5-500 (length) 0.5-20 (diameter)	Traverse cell membrane as 'nanoneedles' without perturbing membrane, Thermal conductivity	[12]
Organic polymers	Colloidal particles of biodegradable polymer matrices, constructed in various design and size	10-1000	Biodegradability, selective drug release, easy surface modification	[13]
Synthetic polymeric NPs	Polymeric NPs prepared from biocompatible and biodegradable polymers. Drug can be dissolved, encapsulated or attached to a nanoparticulale matrix	100-1000	Delivery of higher concentration of drugs, increase stability of volatile pharmaceutical agent	[14]
Iron oxides NPs	Superparamagnetic NPs having spherical nanocrystals of Fe^{2+} and Fe^{3+} core surrounded by dextran or PEG molecules.	1-100	High magnetic susceptibility, Excellent for diagnostic purpose, No in vivo, in vitro aggregation, Easy surface functionalization.	[15] [16]
Metallic NPs Gold NPs Fullerenes Silver NPs	Metal NPs made of gold or silver, have high target specificity	<100	Small particles with large surface area can carry high drug doses, amenable to conjugation to targeting ligand, uniformity in size	[17] [18] [19] [20]
Quantum dots	Semiconductor nanocrystals exhibiting quantum mechanical properties, excellent flourophores.	<10	Wide range of emission frequency, Brighter and more stable signal intensity than organic fluorophores, highly photostable	[21]
Micelles	Amphiphilic surfactant molecules encapsulate both hydrophilic and hydrophobic drugs.	1.5-2.0	Sustained release, target specificity	[22]

Table 2. Therapeutic agents, nanocarries, target and mode of action of anti-HIV nanoencapsulated agents.

No	Therapeutic agents	Nanocarrier	Target cell/tissue	Mode of action	Ref.
1	SPL7013	Dendrimer	Microbicide vagina/rectum	Blocks HIV entry	[75]
2	PSC-RANATES	PLGA nanoparticle		CCR5 internalization	[76]
3	Rilpiravine	Polyethylene polypropylene glycerol`	Macrophage	Non-nucleoside Reverse transcriptase inhibitor	[44]
4	Efavirenz	Mannose poly propyleneimine targeted dendrimer	Monocyte, macrophages	Non-nucleoside reverse transcriptase inhibitor	[49]
5	Nepiravine	Liposome	HBMEC	Alter reverse transcriptase	[53]
6	Lamivudine	Mannose poly propyleneimine targeted dendrimer	Monocyte, macrophages	Reverse transcriptase inhibitor	[77]
7	Zidovudine	Mannose targeted liposome	Lymphnode	Reverse transcriptase inhibitor	[78]
8	Stavudine	Liposome conjugated with mannose	Liver, spleen, Lungs	Reverse transcriptase inhibitor	[65-67]
9	Saquinavir	Transferring-conjugated quantum rods	Brain	Protease inhibitor	[68,]
10	Indinavir	Lipoid E80	Macrophage	HIV protease blocker	[70]
11	Lopinavir	Solid lipid nanoparticles	Intestinal lymphatic vessel	Protease inhibitor	[79]
12	Raltegravir	Gold nanoparticles	lymphocytes, macrophages, astrocytes HBMECs, PBMCs	Integrase inhibitor	[74]
13	DermaVir	Dendritic cell	Lymph nodes	Induce memory T-cell	[80]
14	Phosphorothioate antisense oligonucleotide	Dendrimer Liposome	Lymphocyte	HIV Protection of oligonucleotides from degradation	[81]
15	Gp120 folding inhibitor	Liposome	CD4 antigen		[82]
16	Interferon -α	Nanoparticles	Hepatocyte		[83]
17	F-105	Liposome	HIV +ve cells	Protease inhibitor	[84]
18	Si RNA	Immunoliposomes	Lymphocytes		[85]

6. CONCLUSIONS

Major hurdle in HIV treatment is lack of basic research and extremely slow momentum of new drug discovery. Currently available therapies cART and HAART for the treatment of HIV are unable to remove HIV form reservoirs and also don't elicit memory cells to produce a life time immune response that could remove HIV infected cells. HIV not only displays a wide array of antigens on its surface but also a high rate of incorporation of mutations. The effect leads to high genetic diversity of the virus enabling its escape from T-cell recognition. Nanotechnology has shown some ray of hope as it enables diagnosis at initial stages of infection. It also makes current regimens more effective with attributes like increased drug bioavailability, targeting specificity, sustained release, reduced dosing frequency and least side effects. Development of topical nanomedicines inhibiting the binding of virus to host cell and gene delivering nanocarriers have not been exploited to their full potential in HIV-1 treatment. More emphasis should be on natural compounds such as cortistatin-A and bee venom milletin analogues suppressing HIV-1 replication for the development of anti- HIV drugs. Further multifunctional NPs acting either as theranostic agents enabling simultaneous monitoring and treatment of disease or incorporating more than one drug in a single carrier could be some of the novel approaches which significantly reduce dosing frequency and improve overall patient compliance to the regimen [144-145]. Despite of profuse advantages of nanotechnology, areas like cost efficiency, body metabolism and cytotoxicity still require exhaustive research [9,146]. Further, more randomized trials and *in vivo* and *ex vivo* studies of these nanomedicines are warranted, envisaging these as futuristic ARV candidates in HIV-1 treatment.

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8. ACKNOWLEDGEMENTS

We greatly acknowledge Maharishi Markandeshwar University Mullana (Ambala) for providing the requisite facilities to carry out the said work.