CHAPTER 6

ANTIVIRAL ACTIVITY OF GOLD NANOPARTICLES

6.1  INTRODUCTION

Acquired immune deficiency syndrome (AIDS) is a collection of symptoms and infections resulting from the specific damage to the immune system caused by the infection of the human immunodeficiency virus (HIV). This is a retrovirus of the lenity virus family originally referred as HTLV-III (Weiss1993, Fauci 1993, Barre-Sinoussi 1996). Antiretroviral treatment reduces both the mortality and the morbidity of HIV infection, but routine access to antiretroviral medication is not available in all Countries. It directly and indirectly destroys CD4+T helper lymphocytes (Tb Lymphocytes) and also infects other cell types including macrophages, which are required for the proper functioning of the immune system. When HIV affects the CD4+ cells, the cellular immunity is lost leading to AIDS. HIV classified into HIV-1 and HIV- 2, HIV-1 is a highly infectious and rapidly replicating variant that is primarily responsible for the global pandemic and HIV-2 is a much less virulent species of HIV and largely limited to West Africa. The cases of both HIV-1 and HIV-2 infection have also been reported Worldwide (Gao et al 1999, Keele et al 2006).

HIV-1 entry to macrophages and T helper cells is mediated not only through the enveloping glycoprotein (gp120) with the CD4 molecule on the target cells but also with its chemokine co-receptors such as C-X-C chemokine receptor type 4 (CXCR-4) and C-C chemokine receptor type 5
(CCR-5) (Levy 1996, Dittmar et al 1997). On infection, there is a fusion of the viral and target cell membranes, followed by the viral uncoating, and genomic RNA enters the host cell cytoplasm (Emerman and Malim 1998).

Currently Antiretroviral drugs for therapy involving three to four drugs in combination of non-nucleoside reverse transcriptase inhibitors, protease inhibitors and nucleotide analogues are used to treat the human immunodeficiency virus (HIV). However, antiretroviral drugs do not cure the disease and patients remain infected for life. The HIV transmission is not preventable once if a person was infected with HIV-1 and the problem of developing resistance to a drug remains because HIV reproduces very quickly in the human body and is prone to developing genetic mutations (changes in its genetic makeup). Due to its high rate of replication \(10^9 - 10^{10}\) virions/person/day and error prone reverse transcriptase, HIV can easily develop mutations that alter susceptibility to anti-retroviral drugs.

Scientists are now aiming to use nanotechnology to combining the physical and medical sciences for HIV therapy. With the help of nanotechnology, it is possible to move ahead with traditional medical sciences (Bhattacharya and Mukherjee 2008, Kim et al 2009). A combination of nanotechnology with medical science in the context of dread diseases such as cancer or HIV/AIDS may lead to possibilities that were never imagined. Nanotechnology involves the understanding, design, engineering and fabrication of materials at the atomic and molecular level. Applications of nanotechnology for prevention and treatment of HIV/AIDS have also gained attention in recent years using the most commonly used metal nanoparticles such as gold, silver, titanium oxide and iron (El-Ansary and Al-Daihan 2009). Gold has been used internally in humans for the past 50 years because of its chemical inertness. Radioactive gold is being used in cancer treatment (Molen et al 1979). Gold nanoparticles (AuNPs) are promising candidates in HIV
therapeutics due to their inherent non-toxicity, functionalization and biocompatibility. These property of AuNPs makes them a potential alternative to be looked upon for treatment against HIV.

Gold being inert and relatively less cytotoxic is extensively used for various applications including drug and gene delivery (Connor et al 2005). A recent review of gold nanoparticles and their biomedical applications indicates enormous growth in nanotechnology (Giljohann et al 2010, Shi et al 2010). Gold nanoparticles functionalized with polyethylene glycol or in conjunction with other molecules such as biotin, peptides or oligonucleotides have been synthesized for the biological applications (Oliver et al 2002, Khalil et al 2004) and as a fusion inhibitor for HIV-1 (Kesarkar et al 2012). We used a panel of assays (Lara et al 2010) such as cell based fusion assays, a gp120/CD4 capture ELISA, time of addition experiments to analyze and understand the mode of action of AuNps to inactivate HIV-1. The data from these experiments suggest that AuNps exerted anti-HIV activity at an early stage of viral replication as a viral entry inhibitor.

6.2 AIDS EPIDEMIC

People with AIDS often suffer with infections in lung, intestinal tract, brain, eyes and other organs as well as weight loss, diarrhea, neurological conditions, cancers and certain types of lymphomas. These individuals mostly die from opportunistic infections or malignancies associated with the progressive failure of the immune system. Previous studies reveal that some 60% of HIV-1 infected patients suffer with neuropsychiatric impairment (Ozdener 2005) characterized by cognitive motor or behavioral symptoms. HIV related dementia (Fujimura et al 1996, Melton et al 1997, Goodkin et al 2001) represents the most severe form of HIV related neuropsychiatric impairment and the average survival after diagnosis is six months (Nath et al 1999). The global summary of the AIDS
epidemic till 2010 is presented graphically in Figure 6.1. Worldwide roughly 2.7 million people contract HIV each year as determined by the World Health Organization (WHO), HIV infection and AIDS is pandemic. In 2008, there were 33.4 million people living with HIV and 2 million people died from AIDS. Since its discovery in 1981 some 29 million people have been killed by the disease. It is truly a global illness with new epidemics.

![Bar chart showing the number of people living with HIV, newly infected with HIV, and dying from AIDS-related causes from 2002 to 2010.]

**Figure 6.1** The graphical presentation shows that the number of people living with HIV, number of people infected with HIV and the number of people dying due to HIV infections from the year 2002 to 2010

### 6.3 THE ORIGIN OF HIV, HOW IT CROSSED SPECIES AND IDENTIFICATION OF DISEASE

Some of the most commonly accepted theory states that Simian Immunodeficiency Virus (SIV) was transferred to humans as a result of chimps being killed and eaten or their blood getting into cuts or wounds on the hunter (Wolfe et al 2004). About a million of people in the Belgian
Congo, Ruanda and Urundi were treated with oral polio vaccine during 1950s. It would have resulted that a large number of people becoming infected with HIV-1 due to the contamination of the vaccine with chimp SIV. But the oral administration could not cause HIV, because the lining of the mouth and throat act as good barriers to the virus. It also believed that in 1950s, the African healthcare professionals used unsterilized syringes to multiple patients which would have transferred the virus.

In the early 1980s some of men in New York and California suddenly began to develop a rare opportunistic infections and cancers. It was resistant to any kind of treatment and was not able to cure. All the men were suffering from a common syndrome. The discovery of the Human Immunodeficiency Virus (HIV) proved that it causes AIDS. Now it is accepted that HIV is a descendant of a Simian Immunodeficiency Virus. In February 1999 a group of researchers from the University of Alabama (Gao et al 1999) declared that they had found a type of virus called Simian Immunodeficiency Virus that affects monkeys from chimpanzees and was almost identical to HIV-1 and they claimed that the chimpanzees were the source of HIV-1 and it had crossed species from chimps to humans (Bailes et al 2003). The global summary of the AIDS epidemic till 2010 is given in Table 6.1.
Table 6.1 Illustrates the number of people living with HIV, the number of people newly infected with HIV and the number of people dying due to AIDS and related causes. All the numerical are expressed in millions.

<table>
<thead>
<tr>
<th>Year</th>
<th>Number of people living with HIV (in millions)</th>
<th>Number of people newly infected with HIV (in millions)</th>
<th>Number of people dying from AIDS-related causes (in millions)</th>
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</thead>
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<tr>
<td>2002</td>
<td>29.5</td>
<td>3.1</td>
<td>2.0</td>
</tr>
<tr>
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<td>2.2</td>
</tr>
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<td>2.8</td>
<td>2.2</td>
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<tr>
<td>2010</td>
<td>34.0</td>
<td>2.7</td>
<td>1.8</td>
</tr>
</tbody>
</table>

6.4 STRENGTH OF IMMUNE SYSTEM AND AIDS

According to the Centers for Disease control and prevention (CDC), a person with HIV infection has AIDS when the person’s CD4 cell count falls below 200 (a normal CD4 cell count is 500 or higher). The CD4 cell count is considered to measure the immune status of a person. A person is under a serious condition with any disease and then it is also considered as an AIDS defining illness linked with HIV infection.

6.5 HIV TRANSMISSION, SIGNS AND SYMPTOMS

HIV is a relatively fragile virus and passed directly from host to host through exposure to infected bodily fluids containing the virus. The virus transmission is rapid when virus particles come in contact with torn, broken
and inflamed mucous membranes and also due to exposure either to infected semen, blood, vaginal secretions or breast milk. The virus infects primarily the blood and lymphatic systems specifically targeting CD4+ T cells, dendritic cells, monocytes and macrophages. At the initial stage of infections, the virus spreads rapidly through the immune system and produces symptoms such as fatigue, rashes, fever and lymphadenopathy. This is called latent period and this period lasts from 2 to 20 years with an average of 10 years without any major signs or symptoms and during this period the CD4+ T cell counts drop steadily. When the CD4+ T cell count drops below about 200/microliter of blood then the body becomes susceptible to a wide range of normally harmless opportunistic infections like anemia, fatigue. As the disease progresses more serious complications develop including pneumocystis pneumonia, tuberculosis, gastroenteritis and also the symptoms of headache, confusion, fatigue etc. There is increased risk of cancer and tumor development, when the disease progresses. Also there is a common symptom of memory loss, tremor, swelling in the legs and face, shortness of breath, cough and uncommon fatigue (Balter 1997).

6.6 DIAGNOSIS OF HIV

It is advised to have the early diagnosis to help the patient to live longer by controlling disease multiplication and be less likely to transmit HIV to other people. If it is suspected as HIV infection, a test to detect antibodies to HIV are performed which is a simple and accurate screening test. If the results are positive, then it is confirmed that the person is infected with HIV. Further it is confirmed by the accurate methods like Enzyme linked immunosorbent assay (ELISA) is a one of the screening test to detect HIV antibodies. The rapid screening test is used to detect the antibodies very quickly and to get the immediate result. This kind of test is simpler than ELISA. Another test called Western blot, which is usually performed to confirm when the screening test results are positive. This test is more accurate.
6.7 HIV TREATMENTS

6.7.1 Different Drugs Available for Treatment

Different types of drugs are used to treat HIV infection and are called as antiretroviral drugs. These drugs are to block the enzymes of HIV needs to replicate inside the human cells. These drugs include the following.

Entry or Fusion inhibitors These drugs prevent HIV from entering cells by binds with CD4. To enter a human cell, HIV must bind to a CD4 receptor and one other co-receptor

Reverse transcriptase inhibitors: These drugs prevent HIV Reverse transcriptase from converting HIV RNA into DNA.

Protease inhibitors: These drugs prevent protease from activating certain proteins inside newly produced viruses. The result is immature and defective viruses that do dot infect new cells

Integrate inhibitors These drugs prevent HIV-DNA from being integrated into human DNA

NRTIs (nucleoside and nucleotide reverse transcriptase inhibitors) interrupt the first step that HIV takes to copy itself inside a cell

NNRTIs (non-nucleoside reverse transcriptase inhibitors) also interrupt the first step that HIV takes to copy itself but in a different way than NRTIs.

Drugs that fight HIV have improved the health of many people. HIV medicines can be hard to take and often have side effects. Missing or delaying just a few doses of medicine can lead to the person developing resistance to the drugs, this means that the drugs will stop working. This can
be verified by measuring the CD4 count which begins to return to normal levels. HIV invariably develops resistance to any of these drugs when they are used alone. Resistance develops over a few days to several months of use depending on the drug and the virus.

The treatment is most effective when at least two or three drugs are given in combination. These combinations of drugs are often referred to as Highly Active Antiretroviral Therapy (HAART) (Babiker et al 2000, Gill et al 2002, Porter et al 2003). HAART is used nowadays because the combinations are more powerful than single drug. HAART can delay or prevent AIDS in HIV infected people, thus extending their life time. Widespread use of HAART dramatically increased life expectancy. Undoubtedly, antiretroviral treatment is currently the best option for prolonged and the maximal viral suppression and preservation of the immune system after HIV infection onset (Hammer et al 2008).

6.7.2 Treatment Limitations

Despite the remarkable successes with the current HAART treatment for HIV, there are still various challenges remaining. HIV is able to persist in the human body namely in several reservoir sites. These may be defined as cellular or anatomical locations where a replication form of the virus is persistently harbored with more stable kinetic properties than in the main pool of actively replicating virus (Blankson et al 2002). Also the combinations of antiretroviral drugs have produced unpleasant and serious side effects. Nucleoside reverse transcriptase inhibitors damage mitochondria which help the cells to generate energy. Careful monitoring and changes of drugs can usually prevent serious problems. Drug treatment is beneficial only if the drugs are taken on schedule. Missed doses allow the virus to replicate and develop resistance. No treatments can eliminate the virus from the body, although the HIV level often decreases so much that it cannot be detected.
6.8 NANOTECHNOLOGY FOR HIV/AIDS TREATMENT

The HIV virus can be seen as a biological nanostructure, composed by a host derived membrane, a nucleocapsid and the genetic material in the form of RNA containing three structural genes. As a consequence of constant transcription errors, these viral structures present high polymorphism which leads to mutation thus constituting a major source of antiretroviral resistance development (Levy 2007). The application of nanotechnology to medicine, commonly referred to as Nanomedicine. This involves the use of nanoscale materials for preventive, therapeutic and diagnostic purposes (Zhang et al 2008). A single-dose administration of the drug along with nanosuspension leads to a sustained release over 3 months in dogs and 3 weeks in mice compared with a half life of 38 h for free drugs. These results serve as a proof-of-concept that nanoscale drug delivery may potentially lower dosing frequency and improve adherence.

The indinavir nanosuspensions were loaded into macrophages and their uptake was investigated. Macrophages loaded with indinavir nanosuspensions were then injected intravenously into mice resulting in a high distribution in the lungs, liver and spleen. More significantly, the intravenous administration of a single dose of the nanoparticle-loaded macrophages in a rodent mouse model of HIV brain infection resulted in significant antiviral activity in the brain and produced measurable drug levels in the blood up to 14 days posttreatment (Friedman et al 1993). These studies serve as a proof of concept for indinavir delivery to the brain and the sustained drug levels for up to 14 days, which is important when considering that the half-life of indinavir in its conventional dosage form is 2 h. Various fullerene (C-60) based nano structures, dendrimers and inorganic nanoparticles such as gold and silver have been shown to have anti-HIV activity in vitro (Bosi et al 2003, Kotelnikova et al 2003, Marchesan et al

6.9 STEPS INVOLVED IN THE HIV INFECTION

The infection of HIV with human cell is more schematically explained below.

- Gp120 protein of the HIV virus binds to the CD4 protein of the T-helper cell.
- HIV releases the RNA which is a genetic code of the virus into the cell. For the virus to replicate its RNA must be converted to DNA. The RNA is converted by an enzyme called reverse transcriptase. HIV mutates easily at this point because reverse transcriptase is prone to errors during the conversion of viral RNA to DNA.
- This DNA creates messenger RNA which contains the instructions for making new viral protein.
- DNA is carried into the cell’s nucleus, integrates and binds with the cell’s DNA.
- The virus uses the cell’s natural protein making mechanism to make long chains of viral proteins and enzymes
- RNA and viral enzymes are gathered at the edge of the cell
- HIV virus particles pinch out from the cell membrane without destroying the host cell. To be able to infect other cells, the budded virus must mature. It becomes mature when another HIV enzyme called HIV protease cuts structural proteins in the
virus causing them to rearrange. The schematic presentation of the HIV infection cycle is shown in Figure 6.2.

![Schematic of the HIV infection cycle](image)

**Figure 6.2** The schematic illustration of the steps involved in the HIV infection cycle

### 6.10 MATERIALS

HeLa-CD4-LTR-b-gal cells, HL2/3 cells, HIV-1 cells were obtained from ATCC through R&D-bio, Coimbatore, India. Indinavir, UC781 (NNRTI) and AZT-779, desired quantity of cell free culture were obtained from PSG Institute of Medical Sciences and Research and Sri Ramakrishna Institute of Paramedical Sciences, Coimbatore, India. The viral supernatants was used as viral inoculate.
6.11 EXPERIMENTAL METHODS

6.11.1 Synthesis and Stabilization of Gold Nanoparticles

The gold nanoparticles have been synthesized using a modified Turkevich method (Shankar et al 2005). We have used sodium citrate as a reducing agent as well as a capping agent. In this method 250mL of 0.25mM HAuCl₄ was heated to boiling, then 3.9 mL of aqueous solution of 1% trisodium citrate was added to the beaker containing HAuCl₄ solution under vigorous stirring and the boiling was continued for 15min until it turns a deep red color. The heating mantle was removed and the solution was stirred for an extra 15 minutes. The resulting colloidal solution was allowed to cool overnight after which it was filtered through a 0.2μm membrane filter.

In order to improve the stability of gold nanoparticles, it was conjugated to polyethylene glycol (PEG). About 3 mL of 17nm AuNp was mixed with 212 μl of PEG solution in a 10 mL flask and stirred at room temperature for 1 hour. The solution was then centrifuged at 11,400g for 20 minutes and the gold nanoparticle pellet was redissolved in 2mL of deionized water after the supernatant had been removed. The resulting AuNp-PEG solution was stored at room temperature. PHILIPS electron microscope CM200 with a Operating voltage range of 20-200kv, having the resolution of 2.4 Å is used to investigate the morphology and size distribution of the gold nanoparticles. The size of the gold nanoparticles used for this study was found to be 17nm. The gold nanoparticles were characterized by UV-Vis absorption spectroscopy using a UV-1700 series spectrophotometer to study the peak absorption band. The UV-Visible absorption was measured as 523nm. Zeta potential measurements were performed using a Malvern Instruments Zeta sizer 1000Hs to check the stability of gold nanoparticles as a function of pH. The result shows high stability of gold nanoparticles.
6.11.2 Cytotoxicity Assays

In the cytotoxicity assay experiment, two-fold diluted gold nanoparticles at a desired concentration in the growth medium was subsequently added to 96-well plates having HeLa-CD4-LTR-b-gal cells which express both CXCR4 and CCR5. Micro titer plates were incubated at 37°C in a 5% CO₂ air humidified atmosphere for a further 2 days. Cell viability assay was carried out using a luminescent cell viability assay and the 50% cytotoxic concentration was found based on the percentage of cell survival as compared to positive control. The IC₅₀ of gold nanoparticles was found to be 1.12 ± 0.05 mg/mL.

6.11.3 HIV-1 Infectivity Inhibition Assay

Serial two-fold dilutions of gold nanoparticles were mixed with 10⁵ TCID₅₀ of HIV-1 cell free viruses and added to HeLa-CD4-LTR-b-gal cells with a 0.2-0.5 multiplicity of infection. HIV-1 infection was assessed after two days of incubation by quantifying the activity of the β-galactosidase produced with the Beta-Glo ASSAY System (Promega). The 50% inhibitory concentration (IC₅₀) was defined according to the percentage of infectivity of inhibition relative to the positive control. Gold nanoparticles were tested against Ttropic, M-tropic, dual tropic and resistant isolates and using the indicator cells, infection was quantified by a luciferase based assay. It inhibited all strains of virus showing antiviral potency against these strains. The concentration of gold nanoparticles at which the infectivity was inhibited ranged from 0.05 mg/mL.

6.11.4 HIV-1 gp120/CD4 ELISA

A gp120 capture ELISA was used to test the inhibitory activity of AuNps against gp120-CD4 binding. Briefly recombinant HIV-1₁₁₁₁ gp120
protein (100ng/mL) was pre-incubated for 10 min in absence or presence of serial two-fold dilutions of gold nanoparticles and then added to a CD4 coated plate. The amount of captured gp120 was detected by peroxidase-conjugated murine anti-gp120 MAb. In separate experiments gp120 (100ng/mL) was added to CD4-coated plates pretreated with gold nanoparticles for 10 min period. Before the addition of the gp120 protein, plates were washed three times to remove unbound gold nanoparticles. The plates were washed three times to remove unbound gold nanoparticles before the addition of protein gp120 (Lin et al 2003). The inhibition activity of gold nanoparticles was analyzed in comparison with a known antiretroviral drug Indinavir. Also the antiretroviral drug UC781 known as nonnucleoside reverse transcriptase inhibitor (NNRTI) is used in this cell-based fusion assay.

6.11.5 Time of Addition Experiments

HeLa-CD4-LTR-b-gal cells were infected with $10^5$ TCID50 of HIV-1 cell free virus with a 0.2-0.5 multiplicity of infection, gold nanoparticles (0.1 mg/mL) and (2.5 μM) fusion inhibitor Tak-779 were then added separately at different times (0,1,2,3,…12h) after infection (Neurath et al 2001, Witvrouw et al 2000). Infection inhibition was quantified after 48h by measuring b-gal activity in the Beta-Glo Assay System (Promega).

6.12 RESULTS

6.12.1 Cytotoxicity Studies

HeLa-CD4-LTR-b-gal cells which expressing both CXCR4 and CCR5 was used as models to assess the cytotoxicity of gold nanoparticles. By means of a luciferase-based assay the 50% cytotoxicity concentration (CC50) of gold nanoparticles was defined as 0.12±0.05 mg/mL against HeLa-CD4-LTR-B-gal cells. The cell viability is analyzed against increasing concentrations of gold nanoparticles as shown in Figure 6.3.
Figure 6.3 The result represents the mean viability of three independent experiments and each of these was performed in triplicate. Cell viability was calculated as the percentage of the viable cells compared to the untreated controls.

6.12.2 Inhibition of Env/CD4-Mediated Membrane Fusion

A cell based fusion assay was used to mimic the gp120-CD4 mediated fusion process of HIV-1 to the host cell HL2/3 cells, which express HIV-1 Env on their surfaces and Tat protein in their cytoplasms (effector cells) (Zussman et al 2003, Spira et al 2003). The indicator cells HeLa-CD4-LTR-b-gal can fuse as a result of the gp120-CD4 interaction and the amount of fused cells can be measured with the b-gal reporter gene.

In the presence of a HL2/3-HeLa CD4 mixture, gold nanoparticles efficiently blocked fusion between both the cells in a dose dependent manner (0.05 – 0.8 mg/mL). Known antiretroviral drug, Indinavir and UC781 used as controls in this cell-based fusion assay. In this assay the gold nanoparticles inhibit more than 60% of infection and our results demonstrated that the mode of antiviral action allows the gold nanoparticles to inhibit
HIV-1 infection or fusion better than the known antiretroviral drug UC781 and Indinavir as shown in Figure 6.4 and Figure 6.5 respectively.

**Figure 6.4** Inhibition of the gp120-CD4 interaction using gold nanoparticles and Indinavir. The assay was performed in triplicate.

**Figure 6.5** Inhibition of the gp120-CD4 interaction using gold nanoparticles and UC781. The assay was performed in triplicate.
6.12.3 Gold Nanoparticles Interfere with gp120-CD4 Interaction

The inhibitory activity of gold nanoparticles against the gp120-CD4 interaction was also investigated in a competitive gp120-capture ELISA. A constant amount of gp120 was incubated for 10 min with increasing amounts of gold nanoparticles and the mixture was then added to CD4-coated plate and the amount of gp120 bound to the plate was quantified. Compared with the control (0.0 mg/mL), there was a decrease of over 70% of gp120 bound to CD4 coated plate at the highest dose of gold nanoparticles. As shown in Figure 6.5, there was a significant decrease in absorbance values in the presence of gold nanoparticles (0.05 – 0.8 mg/mL). The gp120 capture ELISA data combined with the results of the cell based fusion assay support the hypothesis that gold nanoparticles inhibit HIV-1 infection by blocking the viral entry, particularly the gp120-CD4 interaction. There is no interference to the absorption signals of the enzyme-linked immunosorbent assay (ELISA) as observed.

Figure 6.6 The degree of inhibition of the gp120-CD4 protein binding was analyzed with gp120/CD4 ELISA capture in the presence or absence (control) of gold nanoparticles. The assay performed in thrice and the error bar indicates the s.e.m.
6.12.4 Time of Intervention

The HeLa cells were infected with HIV-1\sub{IIIb} cell free virus and the addition of gold nanoparticles (0.10 mg/mL) on HIV-1 inoculation at zero time or at the various time point post-inoculation. In our experimental studies, we found that the fusion inhibitor Tak-779 activity started to decline after 3 hr as shown in Figure 6.6. In contrast gold nanoparticles hold their antiviral activities even when added 11 hr after the HIV inoculation. In our result, the gold nanoparticles proved to be a fusion or an entry inhibitor by blocking the viral entry and it effectively reduces the dosing frequency. These results show that gold nanoparticles intervene with the viral life cycle of stages besides fusion or entry. These results shows that the gold nanoparticles potentially reducing the dosing frequency.

![Graph showing anti-HIV-1 activity over time](image)

**Figure 6.7** HeLa-CD4-LTR-b-gal cells were infected with HIV-1\sub{IIIb} and gold nanoparticles (1mg/mL) and antiretrovirals was added at different times post infection. Activity of gold nanoparticles was compared with a Fusion inhibitors (Tak-779, 2.4μM). The assay was performed in triplicate and the data points represent the mean
6.13 DISCUSSION

In this study, the gold nanoparticles were found to be a viral entry inhibitor or exert anti HIV-1 activity at an early stage of a viral replication. The HIV strains found in the human population can differ widely in terms of virulence, pathogenicity and also it differs in its sensitivity to an antiretroviral drug used (Chavda et al 1994). Gold nanoparticles are seen as effective inhibition agents against HIV-1 since they inhibit a varied panel of strains. These peculiar properties of gold nanoparticles exert resistance and reduce the spread of infection. The gp120-capture ELISA data combined with the results of the cell-based fusion assay supported the hypothesis that gold nanoparticles inhibit HIV-1 infection by blocking viral entry, particularly the gp120-CD4 interaction. The time of Intervention experiments further confirmed that the gold nanoparticles act as an entry inhibitors. The gold ions can form a complex with electron donor groups containing sulfur, oxygen or nitrogen that are normally present as thiols or phosphates on amino acids and nucleic acids (Nath et al 2008), they might inhibit the infection by blocking HIV-1 proteins other than gp120 or reducing reverse transcription or proviral transcription rates by directly binding to the RNA or DNA molecules.

The gold nanoparticles have positive surface charge like metal nanoparticles, so they like surface charged gold nanoparticles and V3 loop were not a surface to interact. Here the nanoparticles act as an attachment inhibitors of both gp120 and CD4 rather than as co-receptor antagonists that interfere with gp120 – CXCR4/CCR5 contact (Borkow and Lipidot 2005). The viral absorption assay shows the mechanism of antiviral action of the inhibition of initial stages of HIV-1 cycle. Also cell-based fusion assay supported the hypothesis that gold nanoparticles inhibit HIV-1 infection by blocking viral entry and binding with the protein structures distributed over the viral membrane. We hypothesized that gold nanoparticles not only bind to
gp120 but also modify the viral protein by denaturing its disulfide bonded domain located in the CD4 binding region. The micro chemical reaction between gold nanoparticles and disulfide is not clear, further research work is needed to elucidate the reaction mechanism.

6.14 CONCLUSION

In conclusion, we have established that the fusion inhibition activity of gold nanoparticles is mainly due to the inhibition of the interaction between gp120 and the target cell membrane receptors. Additionally our results demonstrated that the gold nanoparticles act as a viral replication at an early stage by preventing CD4 dependent virion binding, fusion and also blocking HIV-1. Our result demonstrated that the mode of antiviral action allows the gold nanoparticles to inhibit HIV-1 infection or fusion better than the known antiretroviral drug UC-781 and Indinavir. In addition, the fusion inhibitor Tak-779 activity started to decline after 3 hour. In contrast gold nanoparticles retained their antiviral activity even when added 11 hr after the HIV inoculation. In conclusion, gold nanoparticles are effectively at an early stage of viral replication or HIV inhibitor. Also it serves as a proof-of-concept that nanoscale drug delivery may potentially lower dosing frequency. The data presented here open up a window of research to a large and new unexplored area where the gold nanoparticles can be used to target different forms of the HIV virus.