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Abstract #542

Dual Loaded Controlled Release Core-Shell Nanoparticles for Anti-HIV Therapy

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Abstract

Background: Human immunodeficiency virus (HIV) is the world's deadliest infectious disease and progressively suppresses the immune system leading to mortality. Due to length of treatment, high pill burden and adverse effects, patient non-adherence often occurs resulting in viral resistance to current therapeutic regimens. Biodegradable poly(lactic-co-glycolic acid (PLGA) nanoparticles (NPs) coated with chitosan are able to control the release of lamivudine and nevirapine, two commonly used therapeutic agents in resource limited countries.
Common methods of NP fabrication predominantly result in NP sizes >200nm, however to utilize a hollow-fiber pharmacokinetic model to better optimize drug therapies, the size must be <200nm. Furthermore, a decreased size may also improve NP penetration to the brain, and decrease reticuloendothelial system (RES) capture.

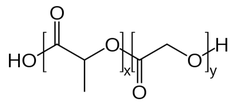
Methodology: Core-shell chitosan-PLGA NPs were fabricated using a flash nanoprecipitation technique using a confined impinging jet followed by solvent evaporation to encapsulate two anti-retroviral drug (lamivudine and nevirapine). NPs were characterized for size and polydispersity using dynamic light scattering (DLS) and nanoparticle tracking analysis; surface charge using a zeta potential analyzer; surface morphology using TEM; and drug dissolution using HPLC. Monocyte derived macrophages (MDMs) that were cultured using standard approaches.

Results: Core-shell NPs display a capsule-like morphology indicating a core-shell structure; a positive zeta potential when chitosan was incorporated during fabrication further confirming the core-shell structure; a decreased size (<120nm); and a controlled release over 24 hours. NP uptake was observed within 30 minutes.

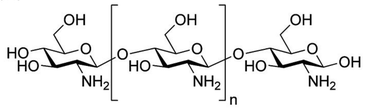
Conclusions: In summary, we demonstrate that chitosan-PLGA NPs are capable of 1) being made to smaller dimensions using commonly available methods in the lab and 2) can encapsulate both lamivudine and nevirapine. These positively charged core-shell nanoparticles deliver therapeutics to viral reservoirs are a revolutionary approach for a more effective, efficient and affordable treatment.

Materials

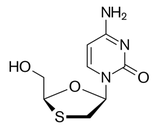
1. Poly(lactic-co-glycolic) acid (PLGA) (50:50, Mw 30,000-54,000 Da) – An FDA approved, biodegradable and biocompatible copolymer. PLGA forms the core of the NP. PLGA is anionic at physiologic pH.



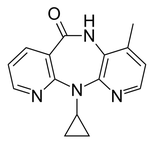
2. Chitosan (Mn 5000 Da)- A linear polysaccharide made by treating crustacean shells with NaOH. Chitosan forms the shell of the NP. Chitosan is cationic at physiologic pH.



3. Lamivudine – A water soluble reverse transcriptase inhibitor (NRTI). Purchased from TCI America, Philadelphia PA.

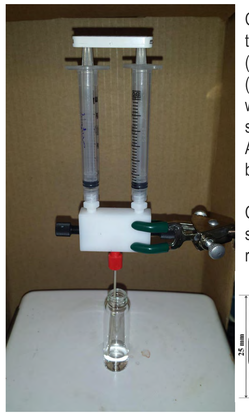


4. Nevirapine -- A well tolerated, non-water soluble, non-nucleoside reverse transcriptase inhibitor (NNRTI). Purchased from TCI America Philadelphia, PA.



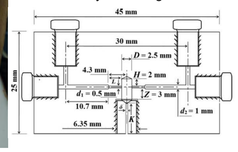
All materials purchased from Sigma Aldrich unless otherwise noted and used without further purification.

Methods



Chitosan-PLGA NPs encapsulating nevirapine, lamivudine and CY5 [a hydrophobic dye used to track the PLGA NP] were fabricated using flash nanoprecipitation (FNP) by a confined impinging jet (CIJ) mixer (Figure 1) followed by solvent evaporation. A 1 mL acetone solution with dissolved PLGA (5 mg/mL), lamivudine and nevirapine (1 mg/mL) and CY5 (1 ug/mL) was mixed against 1 mL of DI water. Both syringes were driven manually and the resulting dispersion was collected in 1 mL of stirred DI water. Syringes were emptied in less than 2 seconds and depressed at the same rate. After FNP, acetone was removed from the NP suspension by evaporation, and NPs were recovered by centrifugation and washed once to remove surface bound drug.

Chitosan was added by adding 10uL 5% w/w polyvinyl acetate (PVA) to 200uL nanoparticle suspension followed by addition of chitosan to a stirred nanoparticle suspension. NPs were recovered by centrifugation and washed once to remove excess PVA and chitosan.



Intensity weighted particle size distribution by dynamic light scattering (DLS) and surface charge (zeta potential) were measured using a Brookhaven 90Plus (Brookhaven Instruments Corporation, Holtsville, NY). Nanoparticle tracking analysis performed using an LM-10 (Malvern Instruments, Westborough, MA).

Figure 1: Flash nanoprecipitation (FNP) device and schematic[4]

Results

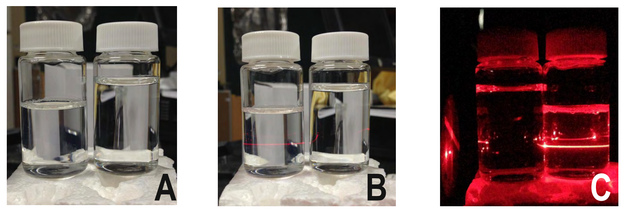


Figure 2: A) NP suspension on left, water on right with laser off; B) Laser on; C) Water on left, NP suspension on right with laser on.

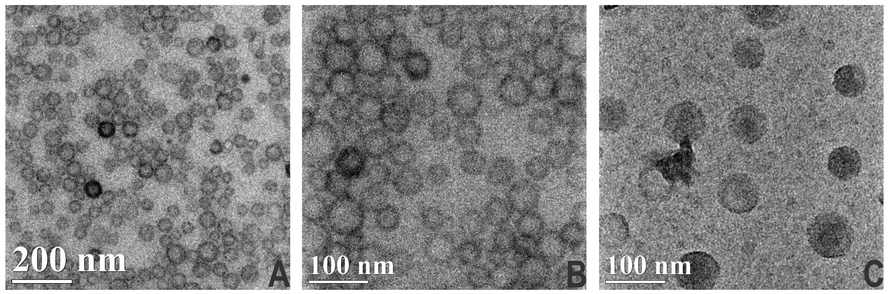


Figure 3: Representative TEM images. A,B) PLGA NPs C) Chitosan-PLGA NPs. Size is less than 100nm with low polydispersity and NPs appear round. Addition of chitosan shell does not dramatically increase the size of NPs and appears uniform.

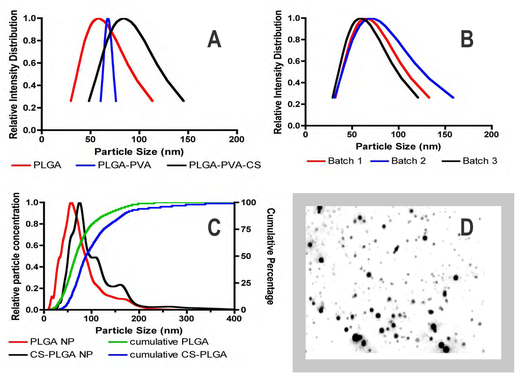


Figure 4: Nanoparticle Sizing results. A) Effect of adding chitosan and PVA to PLGA NPs by DLS using Intensity Distribution. B) Batch to batch variability by DLS. C) Effect of adding chitosan and PVA to PLGA NPs by NTA. D) Single frame image analyzed by NTA.

Note: the same batch was analyzed for Figure 4A and 4C.

	Size (DLS)	Size (NTA)	Zeta
PLGA	58	ND	-30
PLGA-PVA	68	67	-15
PLGA-CS	84	88	24

Results (continued)

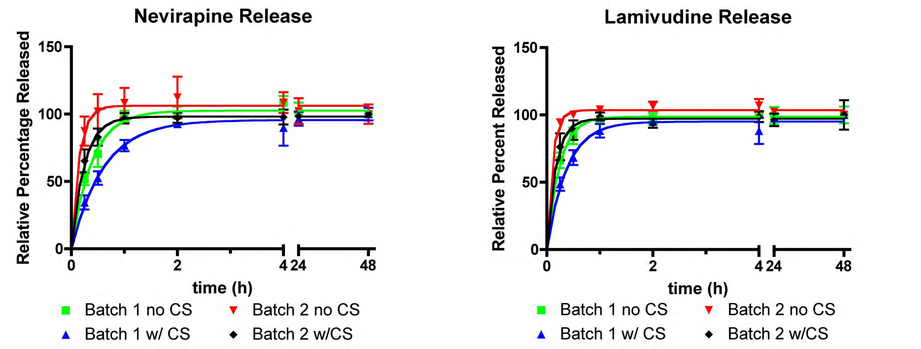


Figure 5: Time release of lamivudine and nevirapine from dual loaded NPs over 48 hours. Drug release data were fit using an exponential equation indicating first order release. Chitosan changes the release pattern of nevirapine and lamivudine.

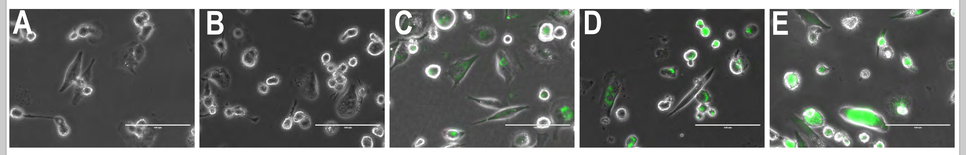


Figure 6: Representative 40x microscopic images cellular uptake. A) control B) free drug 24h C) NP 30 min D) NP 2h E) NP 24h

Summary

Our fabricated Chitosan-PLGA NPs loaded with lamivudine and nevirapine were analyzed by TEM, NTA, DLS, zeta potential and HPLC:

- Transmission electron microscopy (TEM) showed NPs with a core shell configuration (Figure 3).
- Nanoparticle Tracking Analysis (NTA) and Dynamic Light Scattering (DLS) and TEM were used to characterize the effect of chitosan on NP size. NTA, DLS and TEM results were similar. Chitosan coating slightly increases the NP size (Figure 4A and C).
- DLS was used to characterize size variability between three different batches of NPs. The batch to batch variability of NPs is acceptable. The mean NP diameter was 64.8 ± 4.6 nm (Figure 4B).
- Zeta potential of PLGA NPs was -30mV, Chitosan-PLGA NPs was +24mV.
- High Performance Liquid Chromatography (HPLC) confirmed lamivudine and nevirapine released from Chitosan-PLGA NPs over 24 hours.
- Coating NPs with chitosan slows release of both lamivudine and nevirapine (Figure 5).
- Cell uptake occurred within 30 minutes and appeared to saturate after 2 hours (Figure 6).

Conclusions

The resulting dual loaded nevirapine lamivudine NPs offers the potential for several treatment improvements including fewer adverse effects, elimination of high pill burden and the potential to target viral reservoirs.

Future studies include introduction of additional antiretroviral drugs targeting different processes in the HIV life cycle in a single NPs; improved drug encapsulation capacity; and modification of process parameters and composition to effect release pattern, encapsulation efficiency and NP morphology. Additional *in vitro* work to determine NP cellular uptake and localization, intracellular concentration and effective anti-HIV treatment will be performed.

If shown to have good cellular targeting and intracellular concentration, pharmacokinetics and biodistribution studies will be performed.

Acknowledgements

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