A novel alternative "morning after" local administration approach for post-exposure prophylaxis of HIV

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INTRODUCTION

Since 2013 the number of newly diagnosed cases of HIV has practically remained unchanged **(1)**. Current post-exposure prophylaxis (PEP) involves an oral administration, often with considerable side effects. Under WHO guidelines, PEP in a sexual exposure (PEPSE) is limited to only high-risk scenarios **(2)**. The size of the population exposed to low to moderate risk scenarios is unclear.

AIM

The overarching aim of this project is to develop a nanoparticle-based PEPSE with a significant drug loading, for local rectal administration after a low to moderate risk sexual exposure.

METHODS

- Dolutegravir was selected the antiviral of choice. It was then chemically modified, producing a dolutegravir myristate (MDTG) (**Figure 1.**)

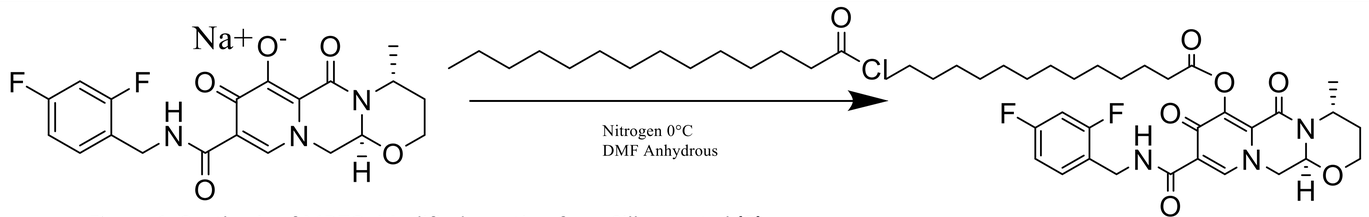

Figure 1. Synthesis of MDTG. Modified reaction from Sillman et al **(3)**.

- A two-step nanoprecipitation system was designed **(Figure 2)**. Three testing conditions were assessed, using different coating masses of mPEG5000-LA100 **(Figure 3).**

- Biocompatibility at 24 hours was on Caco-2 and Raw 264.7 cells**.** Uptake was traced by substituting 10% of the total nano-carrier with a Cy5 Blue labelled PEG5000. **(Figure 3)**.

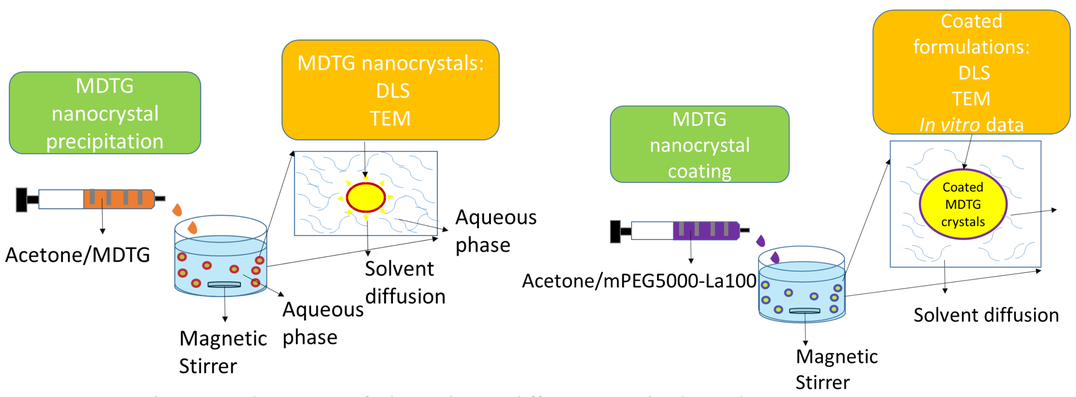

Figure 2. Schematic depiction of the solvent diffusion method used to precipitate and subsequently coat the nanocrystals with mPEG5000-La100.

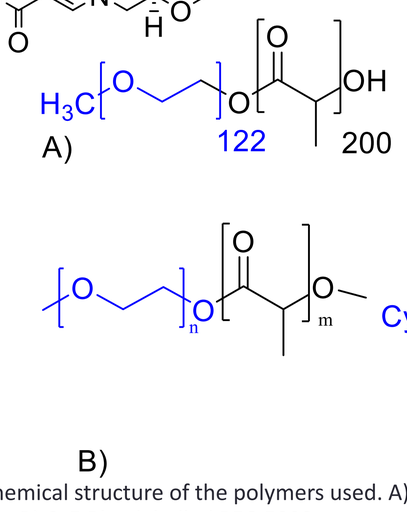

Figure 3. Chemical structure of the polymers used. A) mPEG-LA (122:200) mw 5000 da. B) Cy5 Blue labelled PEG 5000.

RESULTS

- MDTG can be precipitated into an unstable nanocrystal (**Figure 4**).
- All formulations developed contained a significant drug content with a final average of 215nm in size (**Figures 5-6**).
- The coated formulation with 0.22mg was chosen to test further as it carried a significant drug content.
- In vitro data suggests biocompatibility and internalization of the formulation in target cells. Therefore providing potential for drug delivery (**Figures 7-9**).

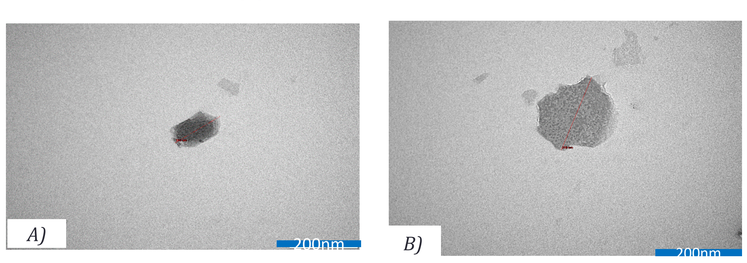

Figure 4. TEM images taken at 8 hours after precipitation of MDTG. *A)*: Image taken at 105K magnification. *B):* Image taken at 87K magnification.

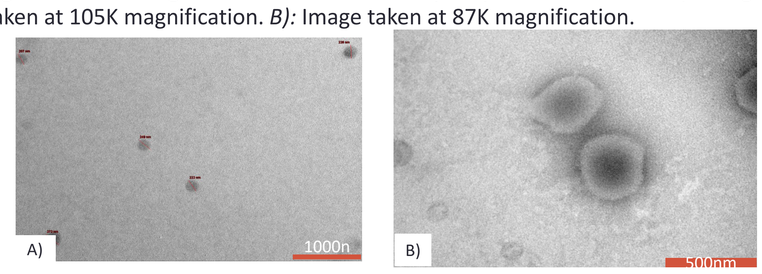

Figure 5. Representative TEM images of a formulation coated with mPEG5000-La100 0.22 mg coating per vial. *A)* and *B)* were taken 12 hours post-coating. *A) Image taken at 16.5K magnification. B)Image taken at 43K magnification.*

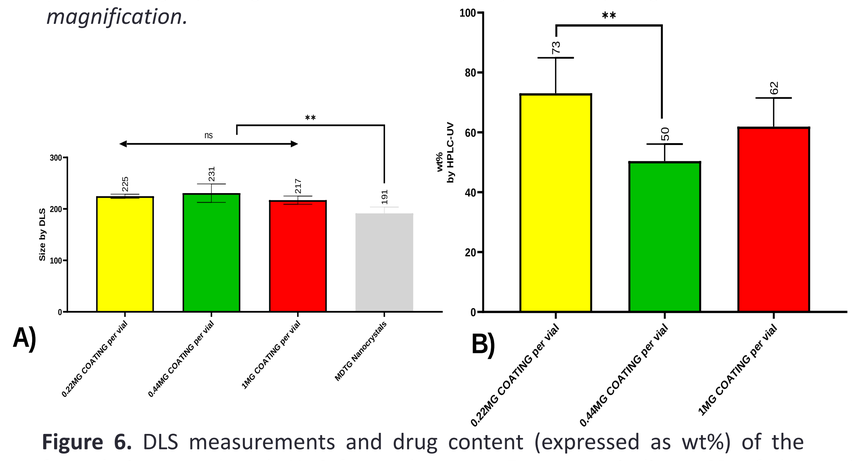

Figure 6. DLS measurements and drug content (expressed as wt%) of the formulations produced. N=6 for each group. Error bars indicate SD of the mean. A) Anova test between groups is significant (p<0.0001). However no statistical difference is found between formulations. B) Wt% of the formulations developed measured by HPLC-UV. The difference between groups is significant after an Anova test (p=<0.0005). Post hoc analysis are displayed on the graph.

Figure 7. Biocompatibility data on Raw 264.7 and Caco-2 cell lines at 24 hours. Panel A shows LDH release on Caco-2 cells. Panel B shows LDH release on Raw 264.7 cells. Panel C shows metabolic activity assessed by Presto Blue on Caco-2 cells. Panel D shows metabolic activity assessed by Presto Blue on Caco-2 cells. Data presented as mean ± S.D (N=3, n=3)

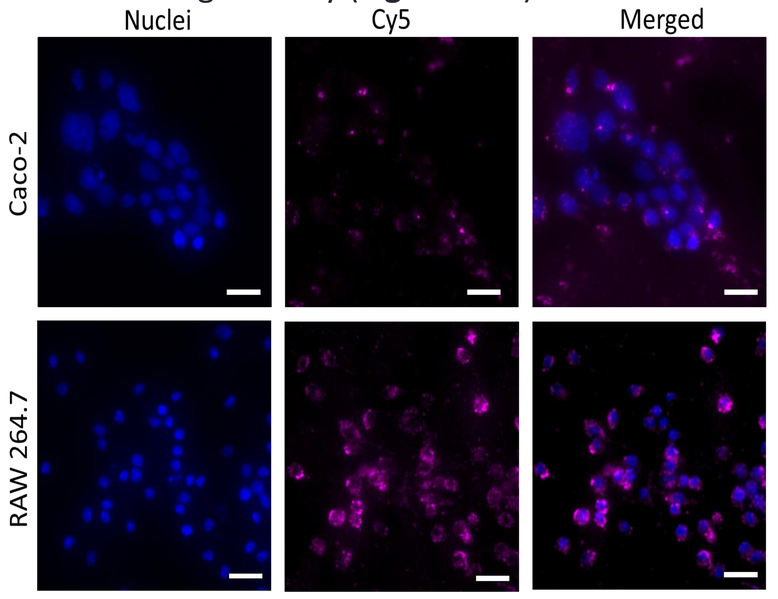

Figure 8. Micrographs of particle internalization in Caco-2 intestinal cells and RAW 264.7 macrophage cells. Particles dosed at 50 ug/ml (polymer concentration) for 24 hours. Scale bar = 50 um.

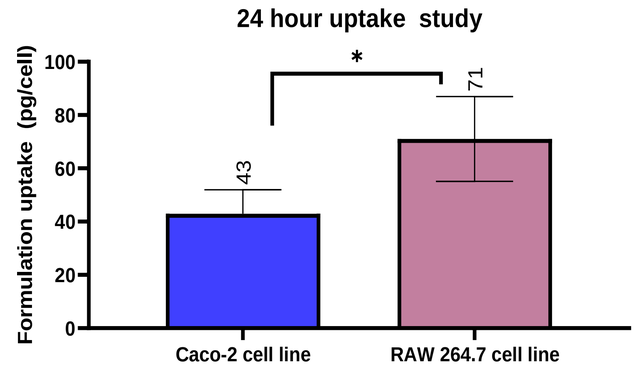

Figure 9. Quantative uptake at 24 hours on Raw 264.7 and Caco-2 cell lines. Data presented as mean ± S.D (N=3, n=3). Unpaired T student test was statistically significant (*, p=<0.05).

CONCLUSIONS/FUTURE WORK

- Due to the 14-carbon chain in MDTG, it can be successfully self-assembled into an unstable nanocrystal through a nanoprecipitation set up. Once in this form, it can be coated with a polymer agent, in a second nanoprecipitation.
- Nanoformulations with a high drug content (above 50% wt%) of MDTG were developed. In vitro data suggests that uptake is higher in Raw 264.7 macrophage than Caco-2 cells at 24 hours. Target cells for this formulation are macrophages and antigen presenting cells, thus the results are encouraging.
- More complex *in vitro* models such as Caco-2/M cells co-culture need to be investigated to provide more physiological relevant uptake data.

REFERENCES

1. HIV/AIDS JUNPo. UNAIDS DATA 2018. UNAIDS/JC2929E. 2018 ed2018
2. December 2018 Supplement to the 2016 Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV infection. Geneva: World Health Organization; 2014 (https://www.who.int/hiv/pub/guidelines/arv2013/arvs2013upplement_dec2014/en/, accessed 6 January 2020).
3. B. Sillman *et al.*, Creation of a long-acting nanoformulated dolutegravir. *Nature Communications* **9**, 443 (2018).