Faculty and Postdoc, Medical, Biomedical and Helath Sciences

Development And In Vivo Testing of Smart Nanoparticles for Enhanced Anti-Cancer Activity and Reduced Cardiotoxicity Associated with Tyrosine Kinase Inhibitors

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Introduction

- Cancer is a growing global problem that is manifested by the uncontrolled division of abnormal cells in part of the body.
- Chronic Myeloid leukemia (CML) is the most encountered subtype of leukemia among adults.
- Anti-cancer therapeutics such as Tyrosine Kinase inhibitors (TKIs) for CML had aid in improving the overall outcomes of patients and increasing their survival rates. However, due to some encountered toxicity of these drugs especially in the heart, the usage of nanotechnology to treat the cancer has been raised.
- Zebrafish is a good model for xenotransplantation of human tumor cells in order to develop a cancer model to study human cancers and testing of the anti-cancer drugs.

Objectives

- To produce smart nanoparticles (PLGA-PEG-PLGA) and define their characteristics.
- To generate a zebrafish xenograft model of CML cancer.
- To test for the toxicity of TKIs and the generated NPs on normal zebrafish.
- To determine the efficacy of the generated NPs as effective anti-cancer drug delivery system by testing them on the zebrafish xenograft model.

Materials and Methods

1. PLGA-PEG-PLGA NPs Synthesis

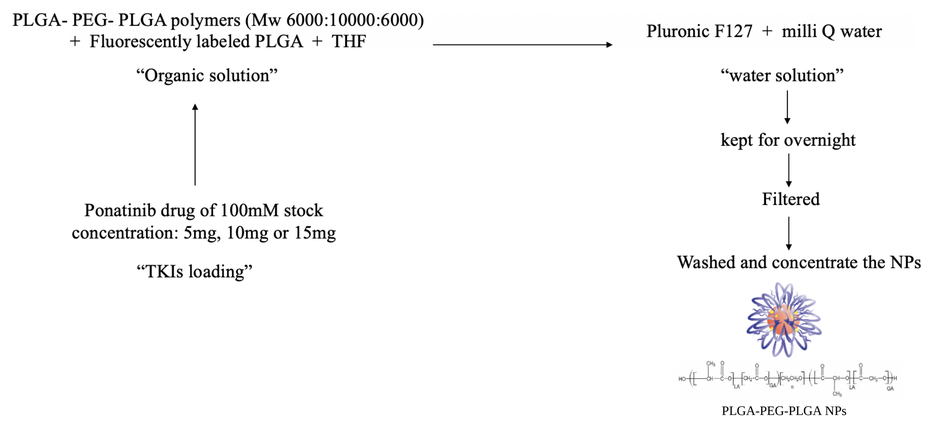


Figure 1: PLGA- PEG- PLGA NPs synthesis. PLGA-PEG-PLGA polymers were measured with a fluorescently labeled PLGA polymers and Tetrahydrofuran was then added with the to have the "Organic solution". The "water solution" was prepared by dissolving Pluronic F127 in milli Q water. After that the organic solution was transferred into the water solution to induce nanoprecipitation and nanoparticles formation. Finally, the dispersion was kept overnight with a magnetic stirrer to evaporate the organic solvent. In the next day, the NPs dispersion was filtered, washed and NPs have been concentrated. And to generate the loaded NPs with Ponatinib drug, 100mM Ponatinib drug stock conc. either a 5mg, 10mg or 15mg from the stock were added to the organic solution.

2. TKIs and PLGA-PEG-PLGA NPs Toxicity

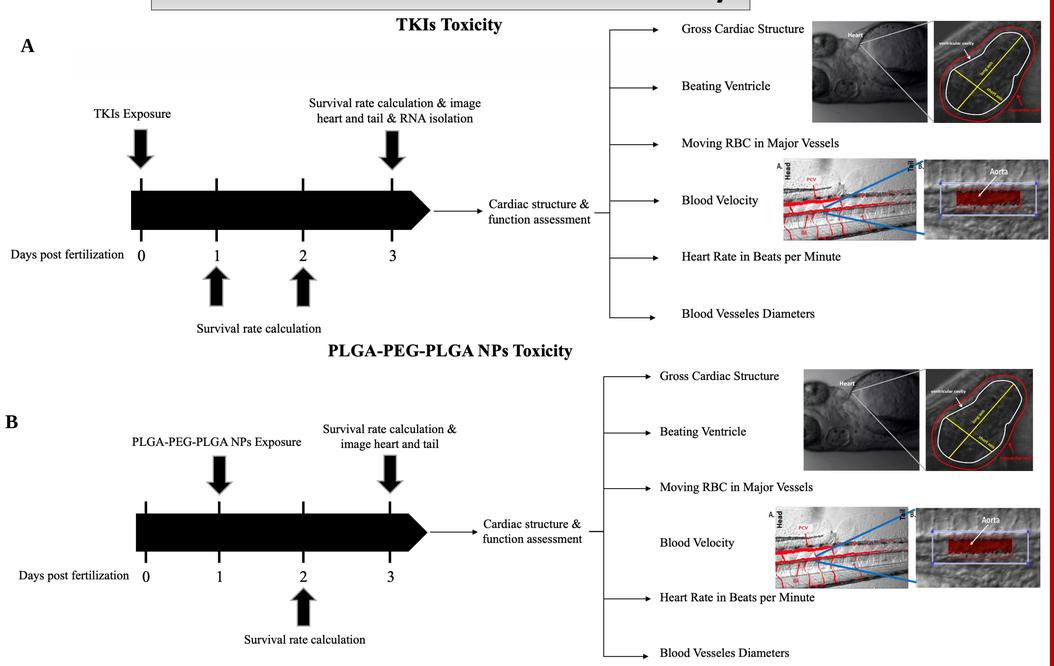


Figure 2 : (A) Cardiotoxicity of Ponatinib and Imatinib. Using zebrafish animal model, the fertilized embryos at 6hpf were placed in a culture plate and exposed to different conc. of the drugs. In the next day and the day after, the survival rate was measured at 2 & 3dpf. At 3dpf the embryos heart and tail were imaged, and the total RNA was isolated. The images and the videos taken for the heart and the tail are then analyzed for the heart gross structure, Beating Ventricle, Moving red blood cells in Major Vessels for the Blood Velocity, Heart Rate as well as for the Blood Vessels Diameters. (B) Cardiotoxicity of NPs by following the same methods as mentioned previously, were the fertilized embryos at 24 hpf or 1dpf are exposed to different conc. of the NPs. And in the next day and the day after the survival rate is calculated and at 3dpf the embryos heart and tail are being imaged for the cardiovascular analysis.

3. Zebrafish Xenograft Model

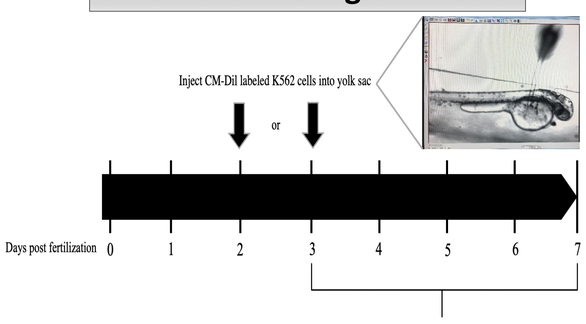


Figure 3 : Zebrafish xenograft model. Fluorescently labeled K562 cell line of the CML were injected into the yolk sac of the zebrafish embryos at either 2 or 3 dpf , and the embryos were then monitored for the cancer cell proliferation until the end point at 7dpf using the fluorescent microscope.

4. NPs exposure to Xenograft Model

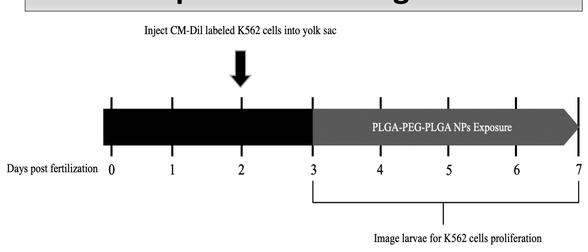


Figure 4 : Xenografted embryos have been exposed to the optimum loaded NPs at 3dpf after the injection of the fluorescent K562 cells. Then the exposed embryos were observed for the cancer proliferation until the end point at 7dpf.

Results and Discussion

1. PLGA-PEG-PLGA NPs 3D Structure

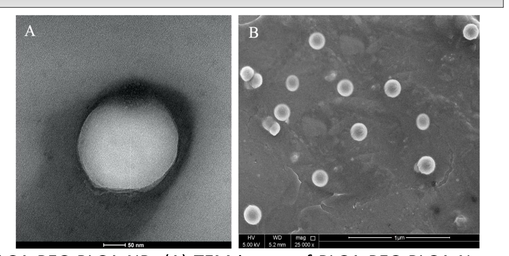
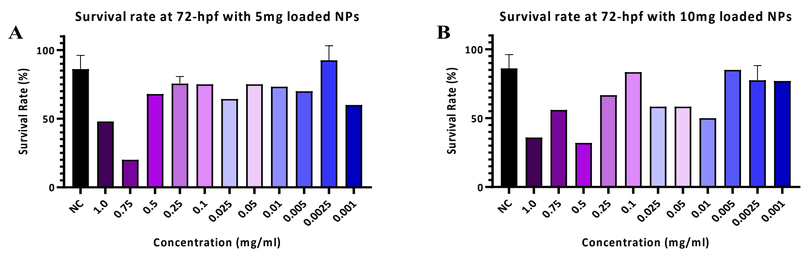


Figure 5 : TEM and SEM micrographs of PLGA-PEG-PLGA NPs.(A) TEM image of PLGA-PEG-PLGA Np on scale bar, 50 nm. (B) SEM image of PLGA-PEG-PLGA NPs on scale bar, 1µm.

2. Loaded PLGA-PEG-PLGA NPs Toxicity



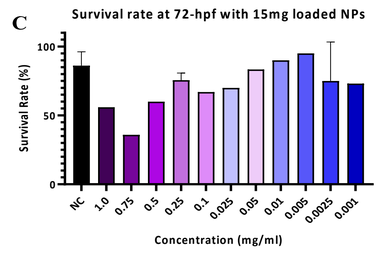


Figure 6 :Loaded PLGA-PEG-PLGA NPs Survival Rate. (A) Survival rate of embryos exposed to different concentrations of 5mg Ponatinib loaded PLGA-PEG-PLGA NPs compared to the NC, at 72 hpf. (B) Survival rate of embryos exposed to different concentrations of 10mg Ponatinib loaded PLGA-PEG-PLGA NPs compared to the NC, at 72 hpf. (C) Survival rate of embryos exposed to different concentrations of 15mg Ponatinib loaded PLGA-PEG-PLGA NPs compared to the NC, at 72 hpf. (*) = $p < 0.05$.

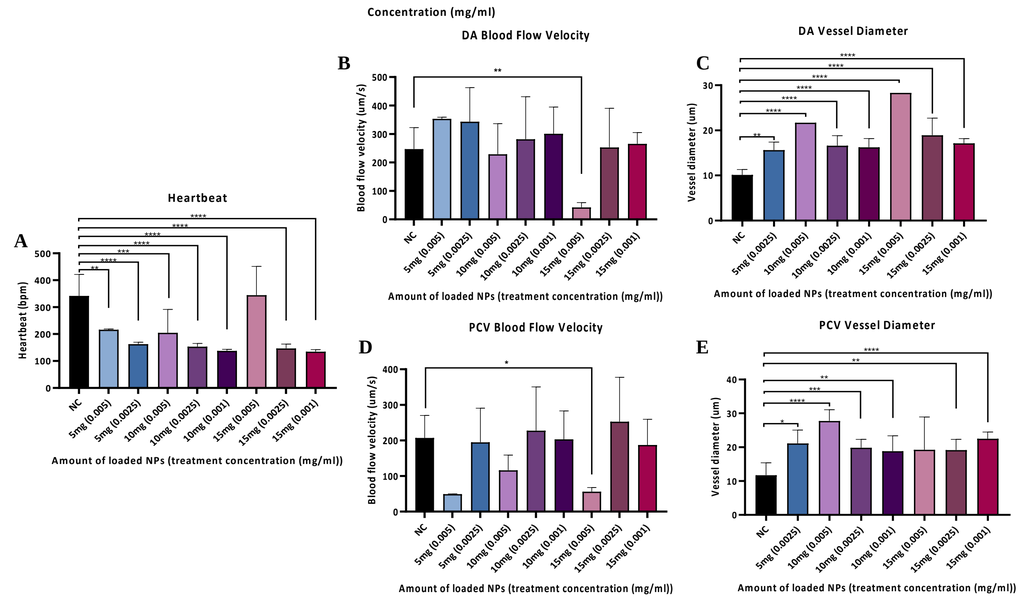


Figure 7 : Cardiac function assessment of loaded PLGA-PEG-PLGA NPs.(A) Heartbeat of embryos exposed to different concentrations of loaded PLGA-PEG-PLGA NPs with 5, 10 or 15mg Ponatinib. (B) DA blood flow velocity of embryos exposed to different concentrations of loaded PLGA-PEG-PLGA NPs with 5, 10 or 15mg Ponatinib. (C) DA vessel diameter of embryos exposed to different concentrations of loaded PLGA-PEG-PLGA NPs with 5, 10 or 15mg Ponatinib. (D) PCV blood flow velocity of embryos exposed to different concentrations of loaded PLGA-PEG-PLGA NPs with 5, 10 or 15mg Ponatinib. (E) PCV vessel diameter of embryos exposed to different concentrations of loaded PLGA-PEG-PLGA NPs with 5, 10 or 15mg Ponatinib. (*) = $p < 0.05$; (***) = $p < 0.001$, (****) = $p < 0.0001$.

3. Zebrafish Xenograft Model

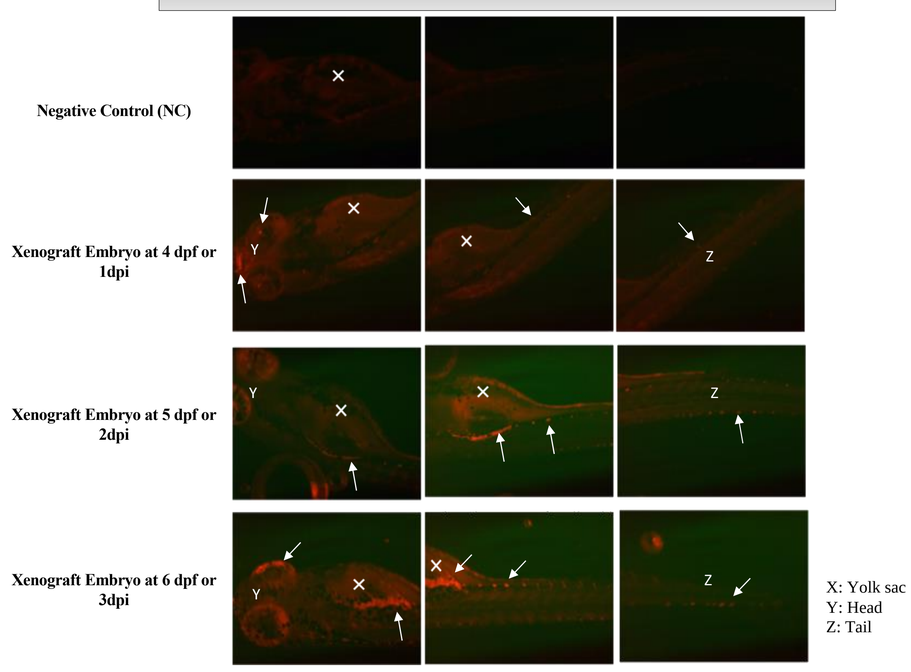


Figure 8 : Zebrafish Xenograft model injected at 3 dpf. Zebrafish screening at 4 dpf to 6 dpf using fluorescent microscopy and investigation of fluorescent K562 cells proliferation (White solid arrows) through out the animal body (Y: eyes, X: yolk sac, Z: tail) using mCherry fluorescence filter.

CONCLUSIONS

- Zebrafish is a good animal model for xenografting and investigating the cardiotoxicity associated with the anti-cancer drugs such as TKIs.
- The lowest concentration of Imatinib and Ponatinib (2.5µM) is the optimum concentration with the least cardiotoxicity and better survival rate.
- The concentrations 0.1 and 0.05 mg/ml of the unloaded NPs are the best with low cardiotoxicity and high survival rate, while 0.001mg/ml of the loaded NPs with 10 or 15mg Ponatinib has shown to be the optimum concentration.
- PLGA-PEG-PLGA NPs could be good candidate for CML treatment, but their cellular internalization should be enhanced.