In Vitro Pulmonary Cytotoxicity of Novel Polymeric Nanocarriers for Drug Delivery
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Purpose
Biodegradable polymers are fueling the development of drug delivery systems due to their biocompatibility, biodegradability, and ease of fabrication. Poly (lactic-co-glycolic acid) (PLGA) is one of such biodegradable polymer and is Food and Drug Administration (FDA) approved for a wide variety of drug delivery systems administered via different routes apart from the pulmonary route. Thus, novel materials are under development for pulmonary drug delivery systems. Poly glycerol adipate-co-pentadecalactone (PGA-co-PDL) is such a new polymer that has been developed and characterized in Liverpool John Moores University (LJMU) laboratory and under intensive investigations and has shown promising results for pulmonary vaccination and macromolecules drug delivery. The aim of this study is to evaluate in-vitro cytotoxicity profile of PGA-co-PDL nanoparticle (NP) carriers in comparison to PLGA NPs for pulmonary drug delivery.

Methods
NPs were formulated by single emulsion-solvent evaporation method using Poly Vinyl Alcohol (PVA) as emulsifier and 1,2-dioleoyl-3-trimethylammonium-propane (chloride salt); DOTAP as a cationic emulsifier, and characterized for size, shape and charge (ζ potential) using the Transmission Electron Microscopy and Zetasizer Nano ZS. In-vitro cytotoxicity was evaluated by Alamar Blue and Reactive Oxygen Species (ROS) assays. The NPs were re-suspended with serum-starve cell culture media prior to cell culture assays. Calu-3 cells were seeded in a density of 40 × 10³ per well in 96 well plates for 48 hours prior to treating with serial concentration of NPs (0.0195-1.25 mg/ml) in triplicate for another 24 hours then colorimetric/fluorometric evaluation using the plate reader.

Results
NPs were successfully produced with a size of 195 ± 3 nm, 212 ± 5 nm for negative (-VE) and positive (+VE) PGA-co-PDL NPs. The PLGA NPs sizes were 148 ± 2 nm, 189 ± 7 nm for –VE and +VE NPs respectively. The shape was smooth spherical particle. The ζ potentials were -13 ± 1.3 mV, +13.9 ± 0.6 mV for –VE and +VE PGA-co-PDL NPs, while for PLGA NPs were -10.9 ± 0.3 mV and +13.7 ± 2 mV. PGA-co-PDL NPs were less toxic in comparison with PLGA NPs confirmed by Alamar Blue assays. The negatively-charged particles were more compatible than positively-charged particles. ROS assay showed that PGA-co-PDL NPs had antioxidant activity in low concentrations and less scavenging activity was detected with the positively-charged NPs (Fig 1).

Conclusion
PGA-co-PDL polymer was successfully formulated into NPs with a suitable size range for pulmonary drug delivery. The results showed a good toxicity profile of PGA-co-PDL NPs in comparison with the PLGA NPs confirming future suitability for pulmonary drug delivery.

![ROS Assay](image-url)

Fig 1. Fluorescence Intensity after 24hrs exposure to both negative and positive charged NP of both polymers by ROS assay (Mean ± SD, n=3).