Effect of physicochemical properties of polymeric nanoparticles on *in vitro* and *in vivo* toxicity

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Statement of Purpose: Polymeric nanoparticles are widely accepted carriers for drug delivery applications due to their tunable physicochemical properties. However, there is no systematic study on effect of physicochemical properties of polymeric nanoparticles on *in vitro* and *in vivo* toxicity. Here, we have developed 12 core-shell polymeric nanoparticles with PLGA, PLA and PCL as core and PVA, chitosan, gelatin and pluronic F68 as shell, of nearly 200nm size with a variation in surface charge and hydrophilicity except PLGA of three different sizes such as 200, 600 and 900nm to study the effect of polymeric nanoparticles on cytotoxicity, hemocompatibility and *in vivo* biocompatibility.

Methods: The PVA and Pluronic F68 nanoparticles were fabricated by emulsion solvent evaporation or nanoprecipitation method and chitosan or gelatin coating was performed by aminolysis followed by adsorption as per our previous report (1,2). PLGA particles were characterized by morphology, size, zeta potential and hydrophilicity. In cytotoxicity, cell death and oxidative stress were evaluated by MTT assay and 2',7'-dichlorodihydrofluorescein diacetate (DCFDA) assay respectively. The hemocompatibility was studied by hemolysis, plasma recalcification, blood clotting, RBC and WBC morphology; nanoparticles and blood interaction by scanning electron microscopy; protein corona adsorptions by SDS page (3). 50mg/kg of nanoparticle suspension in PBS was administered intravenously in mice. The *in vivo* biocompatibility was studied by liver and kidney functions such as plasma albumin, bilirubin, creatinine, blood nitrogen urea, alkaline transferase (ALT), aspartate aminotransferase (AST and alkaline phosphatase (ALP) biochemical assays.

Results: Characterization of fabricated core-shell nanoparticles revealed that they possess spherical morphology, similar size (200±50nm), and dissimilar surface potential i.e., positive (chitosan shell >25mV), negative (pluronic F68 shell, <-22mV), (PV A -10 to-22) or nearly neutral (gelatin shell, 3-5mV) and the hydrophilicity was in the following trends PCL>PLGA>PLA. *In vitro* studies on corneal epithelial (SIRC) cell line revealed that composition of shell strongly influenced cell viability with higher cell death when exposed to positively charged chitosan shell systems as compared to negatively charged (pluronic F68) and neutral (gelatin) shell systems regardless of core composition. Further, increase in concentrations and incubation time of nanoparticles led to increase in cell death, however, increase in size did not have significant effect on cell death. DCFDA data revealed that oxidative stress was strongly influenced by shell composition of the nanoparticles rather than composition of the core. The positively charged chitosan nanoparticles caused higher oxidative stress when compared to negatively charged and neutral particles that corroborated the data obtained from viability studies. The negatively charged pluronic F68 shell systems at higher concentration showed higher hemolysis as compared to positively charged chitosan and neutral gelatin shell systems. Blood and nanoparticle interaction had no effect in RBC and WBC morphology. Also, there was no significant weight loss in mice after 8 days of nanoparticles administration. The *in vivo* liver kidney function test indicated that there was significant difference in the level of AST, ALT and ALP but values were in normal range, however, the level of albumin, bilirubin and creatinine in plasma remain the same (4, 5).

Conclusions: The surface properties have more impact on cyto-and hemo-compatibility than bulk properties. The particles with positive zetapotential have higher cyto- and hemo-toxicity than negative and neutral zetapotential. With dosage of 50mg/kg of nanoparticles, there is no significant toxicological effect on liver and kidney function or on weight loss. The results indicate that systemic *in vivo* application of any polymeric particles made up of with PLGA, PLA or PCL coated with PVA, chitosan, gelatin or pluronic F68 up to a dosage of 50mg/kg is safe irrespective of its physicochemical properties.

References:

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