Multi-functional Polymeric Micelle Delivery System for Drug Resistant Cancer Treatment

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Statement of Purpose: Cancer remains a leading cause of death in the western world. Current therapies include radiation and surgical removal in combination with systemic chemotherapy. However, usage of anticancer drugs has been limited because of their side effects in normal organs and the eventual accumulation of drug resistance by cancer cells[1]. We propose a novel polymeric micelle delivery system that combines targeted chemotherapeutic drug delivery with siRNA directed towards overcoming drug resistance for the treatment of drug resistant cancer. This multi-functional polymeric nanoparticle system consists of three components, 1) amphiphilic copolymers, poly(lactide-co-glycolide)–g-polyethylenimine (PgP), 2) anticancer drug and siRNA targeted to P-glycoprotein (P-gP; protein responsible for multiple drug resistance)[2], and 3) tumor-specific targeting moiety such as an antibody, folic acid, or transferin. Here, we have demonstrated that PgP is an efficient nucleic acid carrier for both pGFP and siGLO in breast cancer cells.

Methods: PgP was synthesized and characterized by ^{1}H-NMR as previously described[3]. The feasibility of PgP as a nucleic acid carrier was evaluated using the Monster Green Fluorescent Protein phMGFP Vector (pGFP (Promega), 2 µg/well) and siGLO Red transfection indicator (siGLO® (Thermo Fisher Scientific), 0.5 and 1 µg/well) in MCF-7 breast cancer cells in 10% serum-containing media. Transfection was performed by complexing pGFP or siGLO-red with PgP at various N/P ratios and subsequently applying the solutions to MCF-7 cells. Complexes of pGFP or siGlo with polyethylenimine (25kDa, branched) at N/P ratio of 5/1 were used as positive controls. At 48 hours post-transfection, cells were collected and transfection efficiency was assessed by flow cytometery; while cytotoxicity was assessed by MTT assay. To evaluate the duration of transfection; time-course study was performed using PgP/pGFP at N/P ratio of 30/1 in MCF-7 cells in 10% serum condition. After transfection, media was changed every two days and the duration of pGFP expression was evaluated by imaging transfected cells with an inverted epifluorescent microscope**.**

Results: MCF-7 cells were transfected using PgP/pGFP in a 10% serum condition. Transfection efficiency of PgP/pGFP polyplexes increased with increasing N/P ratio and was higher than that of PEI at N/P ratio of 5/1 (2.28%) without any significant cytotoxicity at all N/P ratios (Fig. 1 A). Transfection efficiency of PgP/pGFP polyplex at an N/P ratio of 25/1 was approximately 23.8 times higher than that of PEI. Transfection efficiency of PgP/siGlo polyplexes increased with an increasing N/P ratio and was higher than that of PEI at N/P ratio of 5/1without any significant cytotoxicity at all N/P ratios (Fig. 1 B). PgP/siGlo complexes at N/P of 10/1 or above showed greater transfection efficiency than PEI/siGlo complex at N/P ratio of 5/1 without significant cytotoxicity. PgP/siGlo complexes at N/P of 15/1 or above showed similar transfection efficiency with RNAiMax (Invitrogen), which is known as the best transfection reagent for RNAi.

Figure 2 shows the duration of GFP expression in transfected MCF-7 cells using PgP/pGFP at N/P ratio of 30/1 compared to PEI/pGFP at N/P ratio of 5/1 in serum condition. Strong GFP expression was maintained over 20 days.

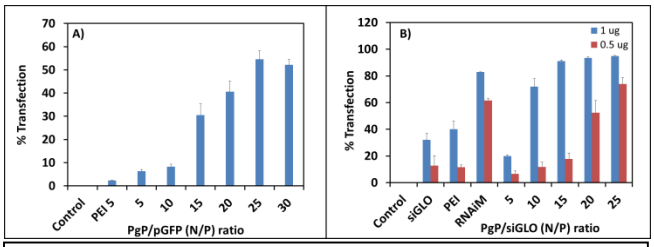

Figure 1: A) GFP expression after transfection of PgP/ pGFP complexes (n=6) B) SiGlo transfection after PgP/siGlo in MCF-7 cells (n=4) in 10% serum condition. Data represent the mean ±SE.

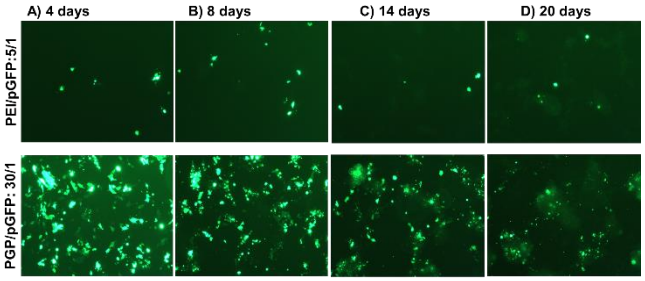

Fig 2. Time-course study of GFP expression in MCF-7 cells with PgP/ pGFP in 10% serum condition. Original magnification 100X, top:PEI/pGFP (5/1), bottom: PgP/pGFP (30/1)

Conclusions: We demonstrated that PgP polymeric micelle is a promising carrier for both plasmid DNA and siRNA capable of transfecting breast cancer (MCF-7) cells in 10% serum condition. We also demonstrated that transfection duration is long lasting. Currently, we are preparing folate (FA) conjugated PgP (FA-PgP) to evaluate the receptor-mediated intracellular delivery of FA-PgP/pGFP complex using MCF-7 cells over-expressing folate receptor and MBA-MB 468 deficient in folate receptors. In the future, we will use siRNA targeting P-gP to knockdown P-gP expression in doxorubicin resistant MCF-7 cells.

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References:

1. Sun et al, Nanoscale, 5:845-859 (2013) 2. Thomas et al, Cancer Control, 10:159-165 (2010), 3. Lee et al. *Trans SFB* p.917 (2010).