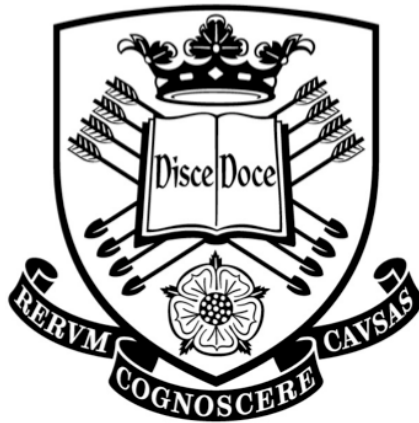


PMPC-PDPA polymersomes-mediated siRNA delivery

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This thesis is dedicated to my teachers and my mum.

Declaration

This thesis is a testimony of the author's work completed in The University of Sheffield, UK, under supervision of Professor Giuseppe Battaglia. This work has not been submitted in whole or any part for any other degrees at this or any other institute.

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Abstract

Polymersomes made from the amphiphilic diblock copolymers, PMPC-PDPA, are proposed to serve as a siRNA carrier with pH-responsive property that provides endosomal escape. The main purpose of this work is to investigate the ability of polymersomes to provide effective intracellular delivery of siRNA into HeLa cells.

Encapsulation of siRNA into polymersomes was performed by pH-switch and electroporation method, both techniques enable siRNA encapsulation. No alteration of polymersomes size and morphology was observed in DLS and TEM. Purification of polymersome was conducted to ensure that no free siRNA or polymer remained.

Intracellular delivery was examined by using fluorescence-labelled siRNA to track the internalisation. Flow cytometry and fluorescence microscope were used to study the cellular uptake of polymersomes and siRNA. siRNA is successfully delivered with the distribution of siRNA signal throughout the cell, with stronger signal compared with Lipofectamine. Kinetic uptake of siRNA suggests that siRNA can be effectively delivered to most cells within 20 hours. In addition, evidence of endosomal escape of siRNA delivered by polymersomes was observed.

Silencing activity of siRNA was determined by qPCR and Western blot, mRNA and protein expression of Lamin A/C as a target gene were not significantly decreased. Cytotoxicity and other cellular response, including pro-inflammatory response and interferon response, were investigated. Polymersomes provide very low cytotoxicity and no pro-inflammatory response, unlike Lipofectamine. Moreover, the gene expression profile of interferon response indicates the possible apoptosis occurrence in Lipofectamine treated cells, but not in polymersomes treated cells.

The information suggests two possible factors that influence the silencing activity of siRNA delivered by polymersomes; the incomplete characterisation of siRNA process and the cellular response from carriers.

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List of Abbreviations

| | |
|----------------|--|
| BCA | Bicinchonic acid |
| cDNA | Complementary DNA |
| DLS | Dynamic Light Scattering |
| DTT | Dithiothreitol |
| dsRNA | Double Stranded RNA |
| EDTA | Ethylene diaminetetracetic acid |
| ELISA | Enzyme-linked immunosorbent assay |
| GFP | Green fluorescence protein |
| GPC | Gel Permeation Chromatography |
| HeLa | Henrietta Lacks |
| HRP | Horseradish peroxidase |
| Hu IL-6 | Human interleukine-6 |
| IFN | Interferon |
| IgG | Immunoglobulin G |
| I κ B | Inhibitor-kappa B |
| mRNA | Messenger RNA |
| MTT | 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| MW | Molecular weight |
| NF- κ B | Nuclear Factor-kappa B |
| m | Metre |
| M | Molar |
| miRNA | Micro RNA |
| PBS | Phosphate Buffer Solution |
| PDPA | Poly-2-(Diisopropylamino)ethyl methacrylate |
| pKa | Acid Dissociation constant |
| PMPC | Poly-2-(Methacryloyloxy)ethyl phosphorylcholine |
| qPCR | Quantitative Polymerase Chain Reaction |
| RIPA buffer | Radioimmunoprecipitation buffer |
| RISC | RNA Induced Silencing Complex |
| RNAi | RNA interference |
| ROI | Region Of Interest |
| rpm | Rounds per minute |
| shRNA | Short Hairpin RNA |
| siRNA | Small interfering RNA |
| TEM | Transmission Electron Microscopy |
| TNF- α | Tumor Necrosis Factor-Alpha |
| U | Enzyme Unit |
| UV-Vis | Ultraviolet-Visible |

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