PMPC-PDPA polymersomes-mediated siRNA delivery

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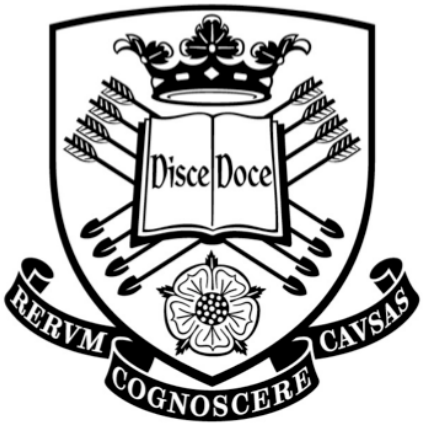

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This thesis is dedicated to my teachers and my mum.

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 publications and presentations

Publications

Peer-reviewed papers

Canton, I., Massignani, M., **Patikarnmonthon, N.**, Chierico, L., Robertson, J., Renshaw, S. A., Warren, N. J., Madsen, J. P., Armes, S. P., Lewis, A. L.,and Battaglia, G. (2013). Fully synthetic polymer vesicles for intracellular delivery of antibodies in live cells. *FASEB J, 27*(1), 98-108.

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Patikarnmonthon, N., Canton, I., Madsen, J. P., Armes, S. P., Lewis, A., and Battaglia, G. (2010, July 11-16). The 43rd IUPAC World Polymer Congress: Macro2010, Glasgow, UK.

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Contents

List of Abbreviations

BCA	Bicinchonic acid
cDNA	Complementary DNA
DLS	Dynamic Light Scattering
DTT	Dithiothreitol
dsRNA	Double Stranded RNA
EDTA	Ethylene diaminetetracitic 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
IκB	Inhibitor-kappa B
mRNA	Messenger RNA
MTT	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MW	Molecular weight
NF-κB	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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