Chapter 1

Introduction

1.1. Background of the study

RNA interference (RNAi) is the biological process where double stranded RNA (dsRNA) can act as the inhibitor of protein translation. The presence of dsRNA with sequence complementary to the target messenger RNA (mRNA) can promote the RNAi mechanism. The process starts from digestion of the dsRNA into the size of about 20 base pairs, the small dsRNA can be called small interfering RNA (siRNA). This siRNA proceeds to further step by interacting with the protein complex, RNA-induced silencing complex (RISC), resulting in separation of siRNA into single stands. RISC unwinds siRNA into single strands while recruiting the target mRNA to the complex and specifically binding to the antisense strand of siRNA. This leads to the target mRNA degradation and consequent inhibition of translation of the associated protein.

RNAi has become an interesting topic for academic researchers and many pharmaceutical companies since the discovery in worms in 1998 (Fire et al. 1998). RNAi paves the way to advanced therapeutic applications, especially for gene therapy. Gene therapy is the use of genetic materials (DNA or mRNA) for the treatment of genetic disorders. To cure abnormalities, the malfunctional genes can be substituted directly by the complete sequence of nucleic acids injected into cells. However, the massive size of those DNA or mRNA molecules can cause problems with cellular internalisation, and their stability. Antisense mechanism, which is the use of complementary DNA (Zamecnik et al. 1978) or RNA (Fire, Xu et al. 1998) to

inhibit the malfunctioning gene, is more favourable due to its smaller size (of only 21 base pairs).

siRNA is widely used to inhibit the protein expression, not only because it has small size (20-22 base pairs) which is easy to manipulate, but also it is an intermediate of the RNAi process that can directly follow to the RNAi pathway to inhibit the translation without any processing required. Although extensive studies on RNAi have been conducted (as shown in *Figure 1.1*), the progress in therapeutic applications of RNAi remains slow. The main problem of siRNA applications is the lack of suitable delivery systems, as siRNA is unable to cross the cell membranes on its own.

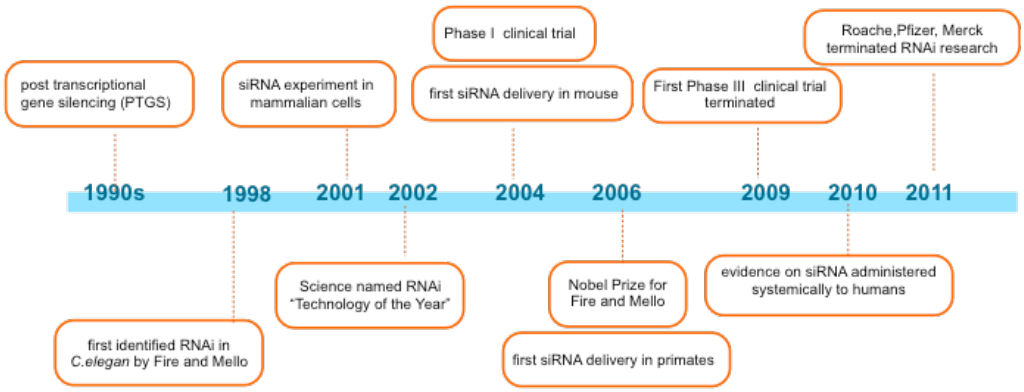

Figure 1.1 : Timeline of discoveries and progress in the field of RNAi.

A potential carrier for siRNA is required not only to promote the intracellular delivery, but also to function as a shield to protect siRNA from enzymatic degradation while avoiding cytotoxicity to the target cells. Studies on different methods, including viral vector, and non-viral based techniques, have been conducted to find the proper RNAi delivery. Even though the use of viral vector provides high transfection efficiency of siRNA with stable expression, this technique generates high immunogenic response and affects cell mortality. In addition, non-viral based techniques have become widely used in siRNA transfection, especially for cationic lipids and polymers. Cationic lipids and polymers can interact with anionic residue of siRNA, resulting in the formation of a complex with net cationic charges on their surfaces that promotes intracellular delivery across the cell membranes. However, the transfection technique is not stable in the environment and the endosomal release mechanism has not fully been clarified.

Recently, our group has introduced the pH sensitive polymeric vesicles, “polymersomes” which provide intracellular delivery for several molecules such as dyes (Lomas et al. 2008, Massignani, Canton, et al. 2010), plasmid DNA (Lomas et al.

2007, Lomas et al. 2010), and antibodies (Canton et al. 2013). These polymersomes are made up of a block-copolymer, the biocompatible PMPC and the pH-sensitive part PDPA which provides for vesicle formation at neutral pH. The block-copolymer becomes soluble in water at pH lower than the PDPA pKa (pH 6.4). Its pH responsive property can be beneficial for disruption in the endosomal compartment as the condition inside endosomes is mildly acidic. The acidic pH inside endosomes triggers the disruption of the polymersomes which disassemble and release the encapsulated molecules causing a rapid increase in osmotic pressure. This increase induces the osmolysis, causing the leak of the active agents from endosomes leading to diffusion of those molecules into the cytosol (Massignani, Canton et al. 2010).

1.2. Hypothesis and objectives of the study

Lack of a suitable siRNA delivery system and previous work on PMPC-PDPA polymersomes lead to the hypothesis that PMPC-PDPA polymersomes can carry, protect and also deliver siRNA into the cells, and siRNA delivered by polymersomes can silence the targeting gene.

The purpose of this study is to use PMPC-PDPA polymersomes as a siRNA carriers for delivery into mammalian cells. This provides an advantage for biomedical research as it would be a novel approach for siRNA delivery with specific properties of siRNA release from polymersomes and endosomes.

The main objectives of this work are:

(i) to improve on the method of siRNA encapsulation,
(ii) to investigate of the intracellular uptake of siRNA,
(iii) to determine the fate and functionality of siRNA after cellular uptake,
(iv) to understand the cellular responses against polymersomes delivery which include toxicity and immune response.

1.3. Overview of Thesis Chapters

This thesis is organised as follows,

Chapter 2: Literature Review

This chapter provides general information and previous findings relating to the studies in this thesis.

Chapter 3: Materials and Methods

All materials and protocols for experimental studies in this thesis were collected in this chapter.

Chapter 4: Results and Discussions I

Optimisation of Polymersomes preparation and siRNA encapsulation.

The first step in this thesis is the polymersomes preparation and ensure the siRNA encapsulation into polymersomes. Validation of polymersomes with siRNA was also performed.

Chapter 5: Results and Discussions II

Cellular Delivery of siRNA

Cellular uptake of polymersomes and siRNA was investigated in this chapter. Fluorescent-labelled polymersomes and siRNA were used to exhibit the effective intracellular delivery of both polymersomes and siRNA.

Chapter 6: Results and Discussions III

Knockdown efficiency and Cellular response of polymersomes mediated siRNA

Knockdown activity of siRNA delivered by polymersomes was determined and compared with available siRNA carrier system, in addition with the studies on the effect of carriers on cellular response.

Chapter 7: General conclusion and Future directions

All findings and conclusions obtained in this thesis are summarised in this chapter. The propose of future work for this research are also suggested.