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Polyplex Based On Polycarbonate Polymers For An Efficient Delivery Of An Anti-Angiogenic siRNA

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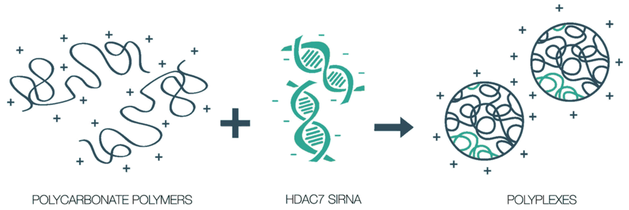
1. INTRODUCTION

- Polyplexes are formed by the self-assembly of **biodegradable polycarbonate polymers** and **siRNA** (small interfering RNA) specifically targeted against **HDAC7** (histone deacetylase 7). The specific inhibition of HDAC7 disturbs the angiogenic process, making it an attractive target for an anti-angiogenic therapy.
- To be effective *in vivo*, polyplexes must meet several **physico-chemical characteristics**. The main characteristics evaluated are the **incorporation** of the siRNA into the polyplexes (by the Quant-iT™ RiboGreen® kit), the **size** (measured by dynamic light scattering), the **charge** (zeta potential measured by laser Doppler velocimetry) and the **buffering capacity**, useful to escape from the endosome (measured by acid-base titration).



- Polyplexes are characterized according to the ratio **polymer positive charge/siRNA negative charge**, called **N/P ratio.**
- The **cellular uptake** of polyplexes with good physicochemical characteristics has been examined in HeLa cells (determined by flow cytometry). Real-time RT-qPCR and western blot have been performed to assess the **expression level of HDAC7** mRNA and protein in treated cells compared to a control.

2. RESULTS AND DISCUSSION

Among different polycarbonate polymers tested, the PTMC-b-PCG-b-PCM showed the most promising characteristics.

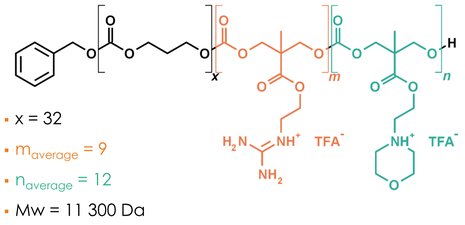


Fig. 1. Structure of the PTMC-b-PCG-b-PCM polymer.

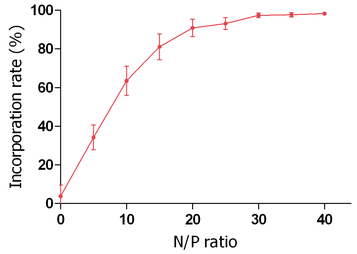


Fig. 2. Incorporation of the HDAC7 siRNA into PTMC-b-PCG-b-PCM polyplexes.

The Quant-iT™ RiboGreen® kit shown an incorporation reaching more than 90% from a N/P ratio of 20. (n=3)

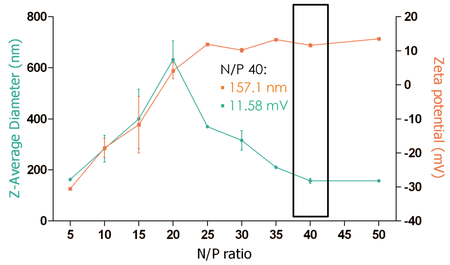
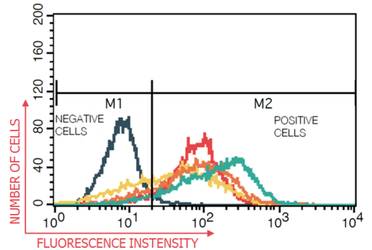


Fig. 3. Evolution of the z-average diameter and the zeta potential of PTMC-b-PCG-b-PCM polyplexes according to the N/P ratio (n=3). The optimal physico-chemical characteristics were reached from a N/P ratio of 40 where we obtained nanoparticles with a diameter of **157.1 nm** and a zeta potential of **11.58 mV**.



▲ PTMC-b-PCG-b-PCM alone
▲ PEI: 95.18 %
▲ PTMC-b-PCG-b-PCM N/P 20: 69.37 %
▲ PTMC-b-PCG-b-PCM N/P 30: 88.95 %
▲ PTMC-b-PCG-b-PCM N/P 40: 95.74 %

Fig. 4. Cellular uptake of polyplexes in HeLa cells was evaluated using the flow cytometry (FACS).

Results shown the distribution of cells according to their fluorescence intensity.

With the polycarbonate polymer, we can observe an increase in the cellular uptake with the increasing N/P ratio, with 69% of transfected cells for N/P 20, 88% for N/P 30 and 95% for N/P 40, a similar result than PEI.

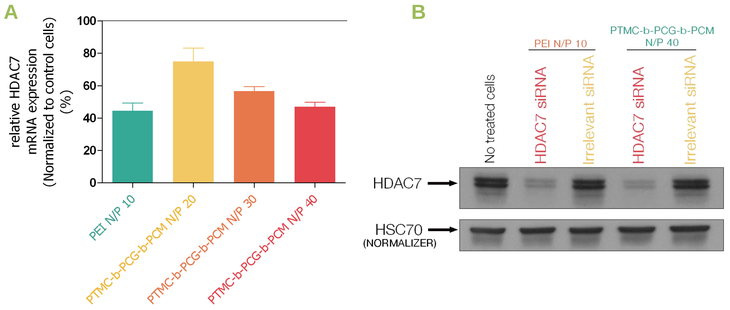


Fig. 5. The efficiency of polyplexes in HeLa was first determined by real time RT-qPCR 48 hours after transfection **(A)**. The relative HDAC7 mRNA expression was normalized to cells treated with irrelevant siRNA. The efficiency of polycarbonate polyplexes increase with the increasing N/P ratio to reach around 45% of relative expression at N/P 40, close to the PEI efficiency. This decrease of the mRNA expression was confirmed by western blot, showing the relative HDAC7 protein expression **(B)**.

3. CONCLUSIONS

To carry and release the siRNA into the cytoplasm of target cells, polyplexes must meet several characteristics: a high incorporation of siRNA, a size and charge compatible with a high cellular uptake and a high buffering capacity to escape from the endosome after the cellular internalization. However, these characteristics are not sufficient. Among various polymers tested, we have shown that the efficacy of polyplexes is increased by the addition of an hydrophobic group on the polycarbonate copolymer. This kind of polymer, like the PTMC-b-PCG-b-PCM, shows promising results compatible with a future potential *in vivo* use.

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