Image-guided Drug delivery by pH-sensitive Polymeric vesicles, polymersomes, by magnetic resonance and optical imaging

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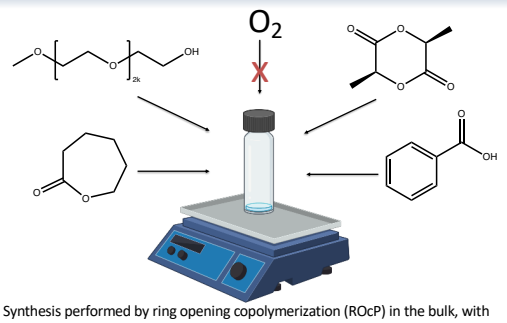
Introduction

New nanocarriers are continually developed to improve the available treatments. Theragnostic nanoplatforms generally combine at least one imaging modality with a drug encapsulation. Here we develop polymer nano-vesicles, also called "polymersomes", which are made of a pH-sensitive polyester shell encapsulating doxorubicin in order to obtain a controlled release of the drug in the acidic tumoral environment. The polymer carrier is composed of an amphiphilic copolymer PEO-*b*-P(CL-*co*-LA) with a statistical hydrophobic block obtained by ring opening polymerization (ROP) of *ε*-caprolactone and *L*-lactide.[1] The interest of LLA comonomer is two-fold: on the one hand to accelerate the drug release through pH decrease in the microenvironment ascribed to degradation into lactic acid, on the other hand to bring semi-crystallinity and thermo-sensibility. The hydrophilic/hydrophobic ratio of the blocks is also a determining parameter to ensure formation of vesicles, therefore we targeted 20%. Self-assemblies are formed by the nanoprecipitation technique with the presence of the drug and of the different imaging modalities, using an aqueous medium (phosphate buffer saline) for biological assays. A bimodal bioimaging system is designed to increase the sensibility by combining optical imaging in the near infrared with the ZW800-1 water-soluble dye, a green indocyanine derivative, together with a paramagnetic contrast agent for magnetic resonance imaging (MRI), one of the most useful modalities to obtain anatomical information. Although it is highly resolutive, MRI suffers from low sensitivity, needing contrast agents (CAs). The clinically approved commercially available CAs are mostly based on gadolinium (Gd^{3+}) complexes, which present a risk of nephrogenic systemic fibrosis for certain patients. That is why the synthesis of complexes based on another magnetic cation, such as manganese (Mn^{2+}), is developed.[2] Manganese ions are potentially less toxic and are naturally present in the body. Further improvements will consist in the optimization of the relaxometric properties of the macrocyclic complexes grafted on polymer nano-vesicles. The final objective is thus to obtain a bimodal nanostructure with encapsulated doxorubicin, and to monitor its release *in vivo* by optical fluorescence imaging (FRI) combined with MRI.

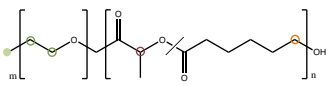
PEO-*b*-P(CL-*co*-LA)

Synthesis



Synthesis performed by ring opening copolymerization (ROcP) in the bulk, with an organic catalyst (benzoic acid)

Results



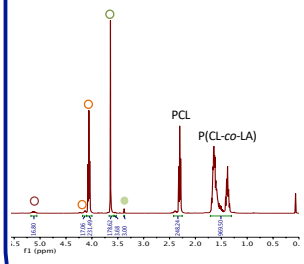


Fig1: ^{1}H NMR (400MHz; CDCl3) of PEO-*b*-P(CL-*co*-LA)

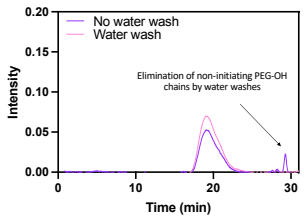


Fig2: SEC trace (RI detector) of the washed and non- washed copolymer

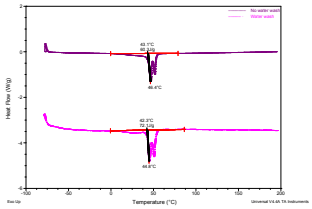
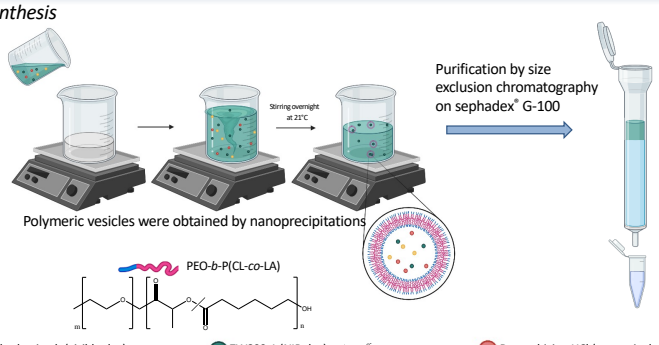
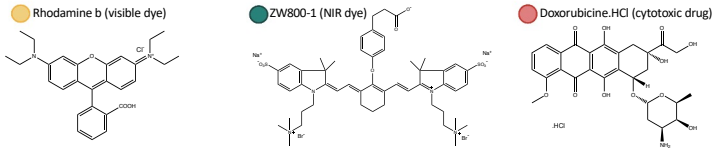


Fig3: DSC curve of copolymers (2nd cycle heating, 10°/min) showing melting peaks at 40°C (PEG) and 48°C (PLLA)

Formation of polymersomes

Synthesis





Results

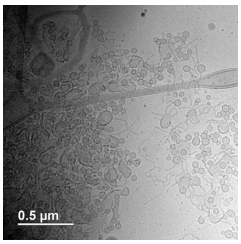


Fig4: Cryo-TEM image of PEO-*b*-P(CL-*co*-LA) self-assemblies in water showing vesicles *

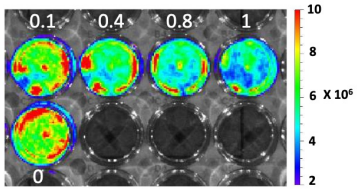


Fig5: Representative bioluminescence (BLI) image of MKN45 gastric cancer cells to obtain their viability as a function of concentration in polymersomes (without doxorubicin loading)

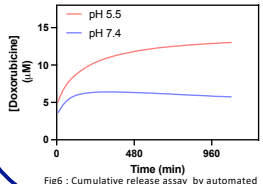


Fig6 : Cumulative release assay by automated dissolution apparatus (SOTAX® USP-4)[3]

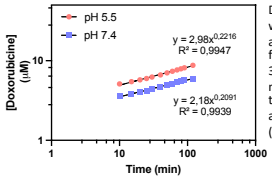


Fig7 : Fits with Peppas-Korsmeyer's law

Doxorubicin release curves were detected from UV-Vis absorbance at 483 nm in flow-through conditions at 37°C and at two pH : in acidic medium (pH 5.5) to mimic tumoral micro-environment and at physiological medium (pH 7.4).

Conclusion & Perspective

Biodegradable amphiphilic copolymers were obtained by organocatalyzed ROcP of ε-CL and *L*-LA units allowing increase of crystallinity and possible auto-catalyzed ester bond hydrolysis by lactic acid release. Drug-loading was optimized, and drug-release assays are under way to demonstrate pH-sensitivity of these polymer drug carriers. First *in vitro* tests on gastric cancer cells allowed to obtain the safety concentration in copolymers. Next experiments consist in assessing IC50 of PEG-*b*-P(CL-*co*-LA) polymer vesicles encapsulating doxorubicin and comparing it to IC50 of the free drug, in order to prepare *in vivo* assays in mice.

Acknowledgement

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References

All sketches were design with BioRender
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[2] Devreux, M. *et al. Inorg. Chem.* **2021**, *60* (6), 3604–3619.
[3] U.ar Bhardwaj, D. J. Burgess, *Int J Pharma* 2010, 388, 287-294.
*Performed by J.-M. Guinier at IMPMC UMR 7590