Polyplex vector for miRNA delivery for the treatment of myocardial infarction

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PhD project: The goal of the project is to design a local drug delivery system from a pullulan-dextran cell-free three-dimensional (3D) scaffold loaded with the nanocarrier, and to evaluate the capacity of this system to deliver miRNA into endothelial cells. Iron oxide NPs will be coated with miRNA and protected with an outer poly(3,3-diméthylmalic acid) (PDMMLA) layer to overcome problems with uncontrolled release associated with current miRNA delivery systems and to control the delivery of bioactive miRNA within the cells. Thus, the two-stage miRNA delivery strategy enables both controllable duration (first stage nanocarrier diffusion from scaffold to cells' surface and cell internalization) and high transfection efficiency (second stage delivery of bioactive miRNA after degradation of the polymer in the cytoplasm).

Workpackages :
WP1 will consist to synthesize cationic PDMMLA through anionic polymerization of achiral α,α',β-trisubstituted β-lactones. The obtained products must combine both the hydrolysable nature of the polyester backbone under physiological conditions towards bio assimilable molecules and properties that can be adjusted by the nature, rate and distribution of the substituents along the polymer chains. Preliminary work has already led to the development of a strategy for the synthesis of racemic lactones from commercial diethyl 2-oxalpropionate. Thus, the synthesis work will make it possible to obtain an amphiphilic copolymer having side chains carrying amino functions (cationic groups). This new functionality on the polyester will be able to interact with the miRNA.
WP2 will consist to synthesize iron oxide NPs, to coat the NP surface with miRNA and finally with cationic PDMMLA. The NP synthesis are already mastered[1]. To coat the NP surface with miRNA and cationic polymer, we will use the direct and one-step complexation already developed for ODN loading. A substantial work will be the physico-chemical characterizations in order notably to quantify the average number of miRNA per NP, miRNA stability and the miRNA release in physiological conditions.
WP3 will consist to impregnate polyplex vector on a pullulan-dextran cell-free three-dimensional (3D) scaffold. A deep characterization of NP-loaded scaffold will be performed.
WP4 aims to study the *in cellulo* and *in vitro* delivery and evaluate the efficiency of miR-155 NP loaded scaffold. We will use fluorescent labeled miRNA 155-5p mimics to assess and quantify the intracellular delivery of NP by confocal microscopy and flow cytometry. The follow-up of the nanocarriers will be performed by magnetic measurements. The biocompatibility will be investigated by MTT assay. The functionality of the miRNA will be assessed firstly with the RILES (RNAi inducible Luciferase expression system) for spatiotemporal detection of miRNA activity developed by Chantal Pichon's lab[2]. This system allows to switch ON the expression of luciferase as function of time and miRNA amount presents inside the cells. HUVEC cells expressing RILES specific to miR-155-5p will be obtained from Chantal Pichon's lab. The scaffold loaded with the nanocarriers bearing different doses of miR-155 will be placed in contact with the cells. Kinetic studies will be performed by measurement the luciferase activity as a read-out of miR-155-5p functionality. Second, we will measure the expression of BACH1 and HO-1 by qRT-PCR and Western Blot. For all experiments, the delivery and efficiency of miRNA NP made with NP alone will be also evaluated. Scaffold loaded with NP complexed with scramble miRNAs will be used as well as negative controls.

[1] Richard, S., Eder, V., Caputo, G., Journé, C., Ou, P., Bolley, J., Louedec, L.; Guenin, E.; Motte, L.; Pinna, N.; Lalatonne, Y. (2016). *Nanomedicine*, *11*(21), 2769-2779.
[2] Simion, V., Henriet, E., Juric, V., Aquino, R., Loussouarn, C., Laurent, Y., & Baril, P. (2020). *J. Contro. Release*, 327, 429-443.