Tripartite Poly-Ionic Complex (PIC) micelles as non-viral siRNA vectors for mesenchymal stem cells.

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Until now, even with an important and fruitful research, the use of cell therapy in a tissue engineering concern still lacks a convincing system applicable in regenerative medicine. The most hopeful systems often rely on 3 main actors: (i) stem cells, (ii) scaffold, and (iii) growth factors to direct the cell differentiation[1]. In this context, we designed a tissue engineering construct for cartilage repair, based on custom made cross-linker free collagen microspheres (µColl) impregnated with TGF-β3 and carrying mesenchymal stem cells (MSC), which allow efficient MSC chondrogenesis both *in vitro* and *in vivo*[2]. However, *in vivo* experiments suggested the late apparition of hypertrophic differentiation markers, which avoid obtaining a fully functional neo-cartilage.

With the goal to more precisely control MSC chondrogenesis, we proposed to use a RNA interference based strategy to inhibit hypertrophic differentiation. However, as primary cells, MSC are known to be difficult to transfect, and require an efficient siRNA vector. Thus, we chose non viral vectors previously used in our lab for primary dendritic cells transfection: PIC micelles[3]. These synthetic vectors were designed to present high nucleic acids loading capacity, high stability in physiological conditions, and enhanced transfection efficiency by disassembling under acidic conditions, such as in endosomes, to release siRNA into MSC cytoplasm. We tested polyelectrolyte complexation of double hydrophilic block copolymers (DHBC) of Polyethylene Oxide and Poly Methacrylic Acid of different molar masses (PEO_x-$PMAA_y$) with Poly-L-Lysine or Polyethyleneimine. The most promising micelles present a hydrodynamic diameter of 46.4 ± 10.6nm with a polydispersity index of 0.210 ± 0.07 and a zeta potential of 9.25 ± 1.10 mV. The encapsulation efficiency has been assessed by electrophoresis and fluorescence correlation microscopy. Using a TAMRA-labeled siRNA, we tracked how micelles are internalized and traffic into MSC (flow cytometry, confocal microscopy). The inhibition efficiency of a significant gene involved in a hypertrophic phenotype, RunX2, was then evaluated by RT-qPCR *in vitro*, exposing interesting transfection results in comparison with the commercial reference Lipofectamine2000®. After these promising results in classic culture conditions, our objective is to load siRNA micelles into µColl in order to enhance transfection efficiency by providing a sustained siRNA delivery, as well as a three-dimensional environment. The characterization of the siRNA release, and the evaluation of interactions between MSC and micelles in 3D will be evaluated. The latest results will be presented regarding the ability of this nano-in-micro system to improve MSC and micelles interaction, and establish the proof of concept of a future gene activated matrix.

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