CHEMISTRY: MOLECULES TO MATERIALS

Institut Charles Gerhardt Montpellier

Gene activated matrix for cartilage repair

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Abstract

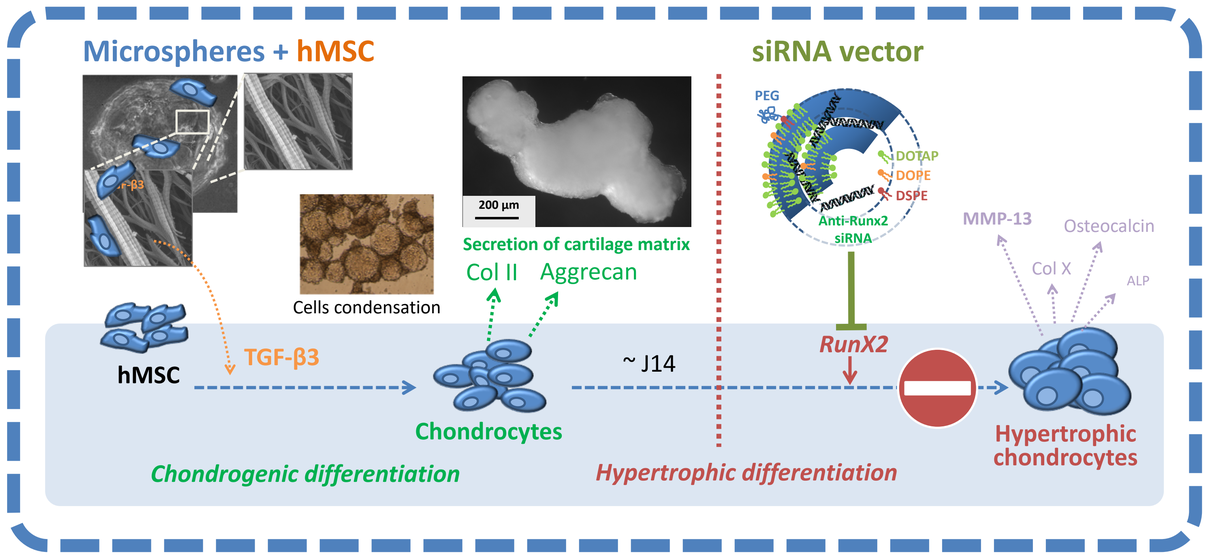
The combination of Mesenchymal Stromal Cells (MSC) with active injectable carriers brings about innovative solutions to current issues in the field of tissue engineering. In particular, repair of adult articular cartilage lesions remains a clinical challenge because of the limited self-healing capacity of cartilage. We demonstrated previously that the open porosity of homemade collagen microspheres allows for the entrapment and progressive release of TGF-β3, which efficiently triggered the chondrogenic differentiation of MSC in vitro and in vivo, and the production of neo-cartilage tissue.

However, one major hurdle in MSC-based therapies for cartilage repair is their late hypertrophic differentiation and subsequent tissue calcification, characterized by the secretion of specific markers such as type X collagen, alkaline phosphatase, osteocalcin and metalloprotease 13 (MMP13). To tackle this challenge, we identified Runx2, which plays a central role in chondrocyte hypertrophy, as the main molecular target to be repressed. Indeed, Runx2 has been widely described to up-regulate the expression of hypertrophic markers. We previously demonstrated that the transient down-regulation of this factor can be achieved with a specific siRNA targeting Runx2.

Here, we intend to deliver siRunx2 from collagen microspheres also used as an injectable support for BM-MSC and as a TGF-β3 reservoir. However, efficient down-regulation of Runx2 with siRNA requires transfection vectors to bring the nucleic acid to its nuclear target within the cells. These vectors will be loaded into collagen microspheres and anchored to the matrix via cleavable peptides.

Strategy

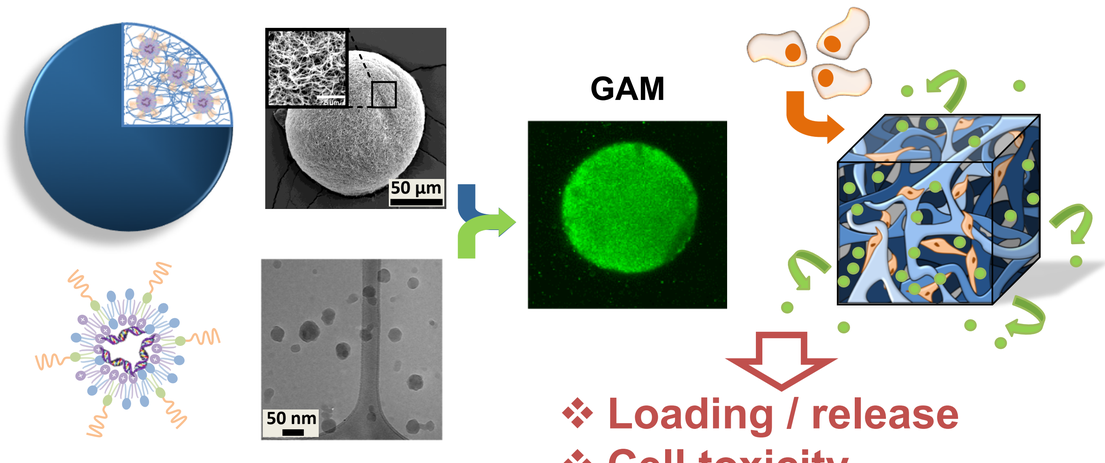
Gene Activated Matrix to prevent hypertrophy in MSC-based cartilage repair



Biological evaluation

❖ Collagen microspheres + TGF-β3

❖ Human BM MSC



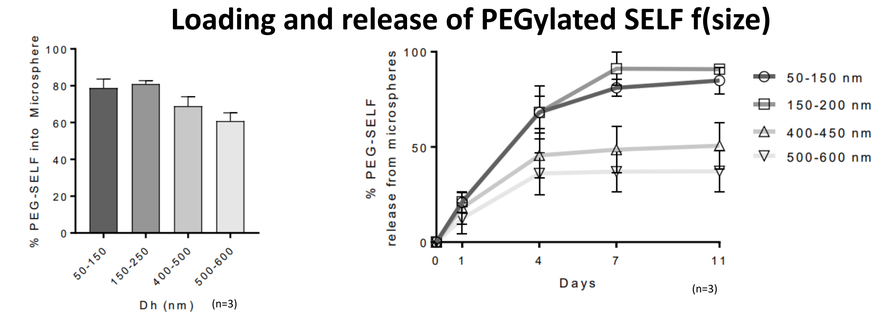
❖ siRNA (Runx2) lipoplexes

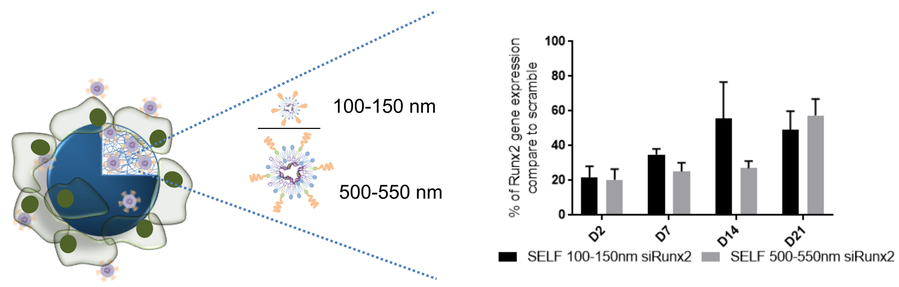
❖ Loading / release

❖ Cell toxicity

❖ MSC differentiation

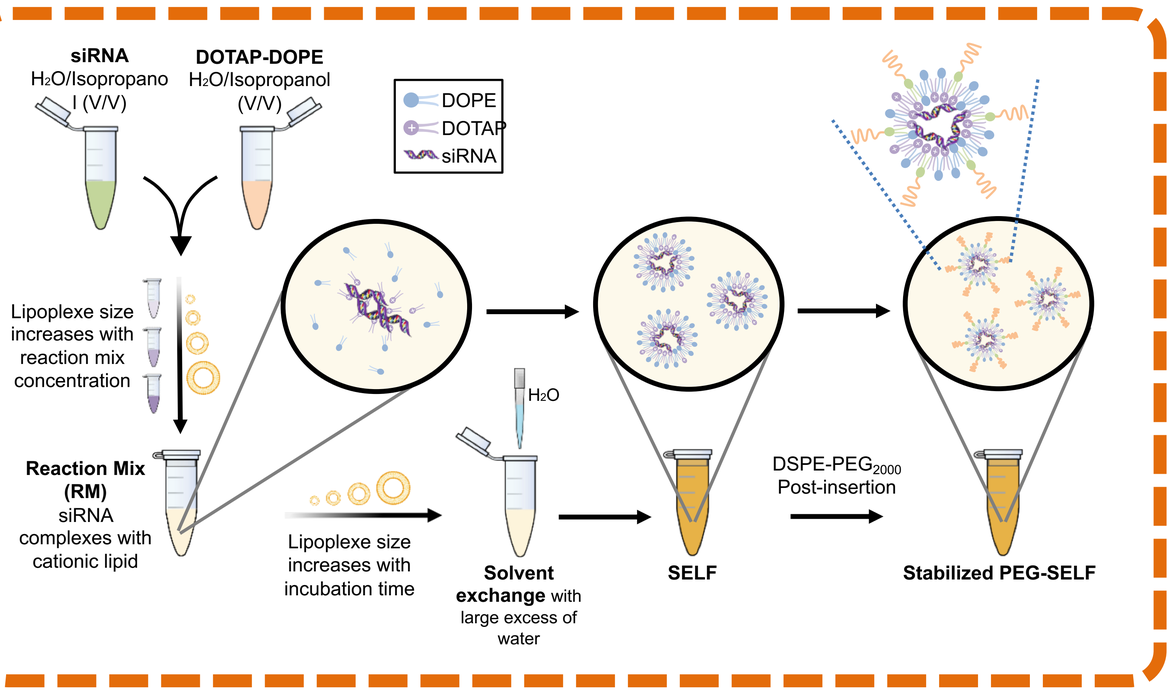
Loading and release of PEGylated SELF f(size)



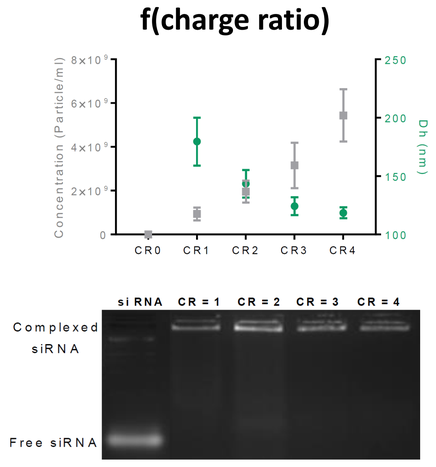


Physico-chemistry

Solvent-exchange lipoplexe formulation

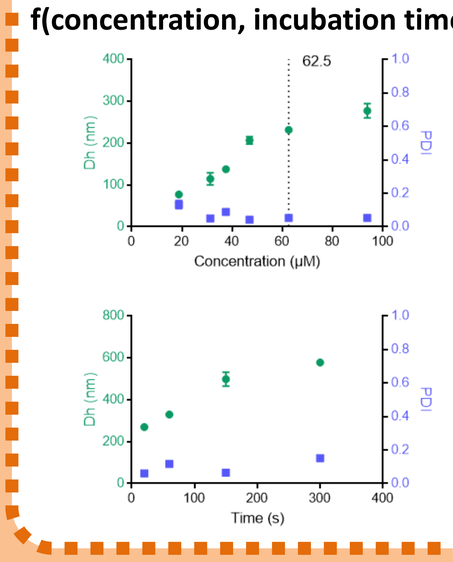


Complexation efficiency f(charge ratio)

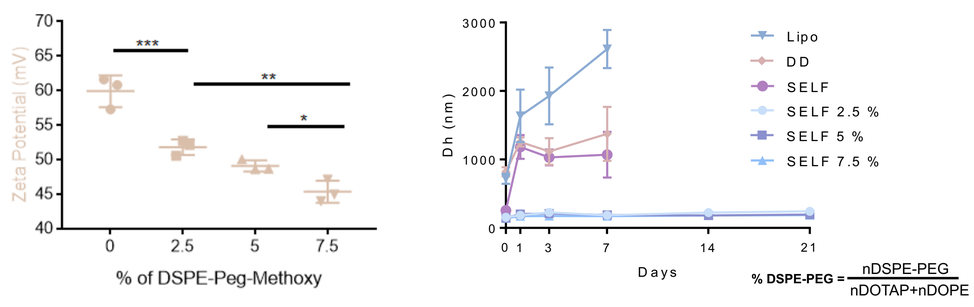


CR = + (DOTAP) / - (RNA)

Size f(concentration, incubation time)



Zeta potential & long-term stability / PEGylation



Missions:

The selected candidate will be in charge of associating the vector with the injectable hydrogel particles. The matrix will be functionalized (i) to increase its mechanical properties and (ii) allow the covalent anchoring of siRNA nanovectors. The functionalization of the nanovector surface will also be studied, to optimize the anchoring. The specific tasks will be adapted depending on the actual advancement of the project. Several characterization techniques will be used for this study: Dynamic Light Scattering, Nanoparticle Tracking Analysis, UV-Vis Spectroscopy, Fluorescence Spectroscopy, Confocal Microscopy, Electron Microscopy, etc…

References :

- Mathieu, M. Vigier, S, Labour, MN , Jorgensen, C, Belamie, E* and Noel, D*, Induction Of Mesenchymal Stem Cell Differentiation And Cartilage Formation By Cross-Linker-Free Collagen Microspheres 2014 European Cells & Materials 28 : 82-97.
- S. Raisin, M. Morille, C. Bony, D. Noel, J.-M. Devoisselle and E. Belamie Tripartite polyionic complex (PIC) micelles as non-viral vectors for mesenchymal stem cell siRNA transfection, Biomater. Sci. 2017, 5, 1910-1921.