Injectable hydrogels for microRNA delivery to promote direct cell reprogramming in cardiac regeneration

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INTRODUCTION

Cardiac regeneration post- myocardial infarction (MI) represents a worldwide clinical challenge due to the poor regenerative capabilities of heart tissue. After MI, cardiac extracellular matrix undergoes significant remodeling leading to the formation of a fibrotic scar populated by cardiac fibroblasts (CFs), non-contractile cells. Regenerative medicine approaches are currently being explored to replenish the population of cardiomyocytes (CMs) and re-establish the functionality of injured myocardium. In particular, the direct reprogramming of CFs into induced CMs using a combination of four micro-RNA (miRNA), named miRcombo, has shown encouraging results both *in vitro* and in mouse models[1]. MiRcombo encapsulation in lipoplexes (LPs) can highly improve miRNA cell uptake and transfection efficacy. Furthermore, LPs embedding into an injectable hydrogel could improve their *in situ* delivery to the pathological cardiac tissue. Algysil LVR, an alginate-based material, has been investigated in clinical trials to favor cardiac tissue remodeling after MI[2]. However, Algysil LVR is not cell adhesive and is a permanent device due to alginate poor *in vivo* degradability. To overcome these hurdles, an oxidised formed of alginate, alginate dialdehyde (ADA), was herein explored as a delivery system[3].

In this work, an ADA-based injectable hydrogel was developed able to encapsulate and release miRNA-loaded LPs. To improve hydrogel cell adhesive properties, hydrogel composition was optimized through functionalization with a chemically-modified gelatin (Gel-M).

EXPERIMENTAL METHODS

ADA was prepared by partial oxidation of alginate with sodium metaperiodate and characterized using MAS solid-state NMR spectroscopy and via the iodine–starch test to determine the degree of oxidation[3]. ADA hydrogels were obtained by ionic crosslinking with calcium ions using a double syringe mixing system and characterized rheologically. Novel LPs containing miRNA were prepared *via* spontaneous electrostatic interaction and physically encapsulated in the ADA hydrogels[4]. Release studies of a Cy5-siRNA-loaded LPs from ADA hydrogels were conducted in distilled water at 37°C and estimated by plate reader analysis.

A modified gelatin (Gel-M), able to react with ADA was produced and characterized for its functionalization degree by 2,4,6-Trinitrobenzene Sulfonic Acid (TNBS) assay[5]. ADA/Gel-M hydrogels were prepared varying the polymers weight ratios (%w/w) (from 70-30 to 30-70, ADA: Gel-M), keeping constant the final polymer concentration (%w/v). Adult human CFs (AHCFs) were cultured on ADA/Gel-M hydrogels for 1 and 7 days, and the adhesion of DAPI-stained cells was evaluated by fluorescence microscopy. The analysis of the release of miRNA-loaded LPs from ADA/Gel-M hydrogel is in progress.

RESULTS AND DISCUSSION

ADA with an oxidation degree of 40% was produced from alginate with an average yield of 75%. The presence of aldehyde groups was confirmed by ATR-FTIR and ^{13}C MAS NMR spectroscopy. Tailored calcium ions and polymer concentrations allowed to obtain physical hydrogels with viscoelastic properties similar to Algysil LVR. Novel miRNA-loaded LPs were entrapped into ADA hydrogels without altering rheological properties. Cy5-siRNA, a model fluorescent oligonucleotide loaded in LPs was completely released from the hydrogel in 24h. To improve cell adhesive properties of ADA hydrogels, Gel-M was prepared with an average yield of 80% and with an average degree of functionalization of 14%. Injectable hydrogels with different viscoelastic properties were developed exploiting the possibility to create chemical crosslinking between ADA and Gel-M. Biocompatibility studies with AHCFs showed that the incorporation of Gel-M significantly increased CFs adhesion compared to ADA-based materials.

CONCLUSION

A novel strategy for cardiac regeneration was investigated based on the *in situ* delivery of miRNA-loaded LPs through ADA-based injectable hydrogels to promote myocardial regeneration. The incorporation of Gel-M into the hydrogel matrix supported CFs adhesion. We are currently investigating the release of miRNA-loaded LPs from this novel ADA/Gel-M matrix.

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