Sustained Release HGF Monospheres for Treating Ischemic Heart Disease: A Proof of Concept in Infarcted Rats

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Purpose
Chronic Heart Failure (CHF), caused by myocardial infarction and subsequent ischemic heart disease (IHD), is a terminal disease with an annual mortality rate of ~18%. The sole current treatment is heart transplantation. We propose treatment of IHD, and prevention of CHF, by site-specific delivery of growth factors (GF) to the ischemic heart region via intra-coronary administration of GF-loaded microspheres of a uniform size of 15 μm. Due to their size, the microspheres will become trapped in the small capillaries of the ischemic region, allowing localized release of GF into the ischemic tissue and tissue regeneration. Hepatocyte Growth Factor (HGF) has been reported as a candidate GF for treatment of IHD. Here we report on the development of monodisperse microspheres (monospheres) composed of hydrophilic SynBiosys® Pro multi-block copolymers for sustained release of HGF. Our aim was to test the efficacy of intra-myocardial (IM) administration of HGF monospheres in infarcted rats.

Methods
SynBiosys Pro [PCL-PEG-PCL]- b-[PLLA] multi-block copolymers with 25 wt% of PEG1000 (PCL05) or 22.5 wt% of PEG3000 (PCL01) were synthesized using similar procedures as previously reported. HGF monospheres were prepared by membrane emulsification (Microsieve Emulsification™, Nanomi B.V.) using a double water-in-oil-in-water emulsion solvent extraction/evaporation process and a membrane with uniformly sized pores of 7 μm. HGF monospheres were characterized for particle size distribution by Coulter Counter and for HGF content by extraction. In vitro release of HGF was measured in 10mM TRIS pH 7.4 at 37°C. RP-UPLC was used to quantify HGF concentration of extraction and release samples. Rats were infarcted by permanent ligation of the left descending coronary artery and 72x10³ HGF monospheres were intra-myocardially injected just after the tissue became pale. Cardiac function was evaluated by echocardiography measurements. Changes in Fractional Shortening were calculated as: ([LVDd-LVDs]/LVDd) x 100, where left ventricular dimensions (LVD) were measured at diastole (d) and systole (s). Results are presented as the mean ± SD, * p < 0.05.

Results
Uniformly sized HGF monospheres with a size of ~15 μm were successfully manufactured of 50/50 w/w blend of PCL01/PCL05. HGF could be encapsulated into the monospheres with HGF load of 2 wt%. HGF was released structurally intact from the monospheres in vitro without any burst release for up to 2 months (Figure 1). The ATM HGF monospheres were tested in vivo in rats. 72x10³ monospheres were injected into the heart muscle of infarcted rats. The fractional shortening improved significantly after 30 days in HGF treated animals when compared to control animals injected with placebo monospheres (Figure 2). This improvement was maintained until at least 45 days.

Conclusion
We have shown that monodisperse microspheres composed of a blend of SynBiosys Pro polymers PCL01 and PCL05 are suitable for sustained release of structurally intact and bioactive HGF for up to 2 months. In vivo study in infarcted rats showed significantly improved cardiac function upon intra-myocardial administration of HGF monospheres. The developed HGF monospheres are currently being evaluated in an acute myocardial infarction pig model to assess their safety and efficacy with respect to regeneration of ischemic cardiac tissue.