

## Bioengineered Controlled Delivery System for the Treatment of Acute Liver Failure

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**Statement of Purpose:** Acute liver failure (ALF) is a medical emergency frequently occurring in otherwise healthy individuals as a consequence of both intentional and unintentional drug overdoses and interactions, hepatitis or without an obvious cause. Historically mortality rates in ALF have been over 80%. Even with supportive intensive care treatment, liver transplantation is often necessary, and overall mortality remains over 30%. The goal of this study was to investigate clinically relevant delivery systems for hepatocyte growth factor (HGF) and hepatocarcinoma-intestine-pancreas/pancreatic-associated protein (HIP/PAP), two paracrine hepatic growth factors that promote the proliferation and viability of liver cells and have hepatoprotective effects. Here we present progress towards the development of a polymeric controlled delivery approach for highly efficient delivery of protein growth factors that improve recovery in a mouse model of acute liver failure.

**Methods:** Recombinant human hepatocyte growth factor (rhHGF) and hepatocarcinoma-intestine-pancreas/pancreatic associated proteins (rhHIP/PAP) were encapsulated using modified alginate ionic crosslinking, poly(lactic-co-glycolic) acid (PLGA) and poly(hydroxybutyrate-co-valerate) (PHBV) double emulsion methods. Particles were characterized using scanning electron microscopy and dynamic light scattering and zeta potential measurements. *In-vitro* release profiles were obtained by incubation in complete media at 37°C, with the amounts of released and encapsulated protein determined using appropriate ELISAs. Primary adult mouse hepatocytes were isolated using the two-step collagenase perfusion technique and cultured *in-vitro* on Matrigel sandwich plates. Hepatocyte viability was determined using the CellTiter Blue viability assay and LDH release. P-450 activity and albumin secretion were determined using a luminescent substrate assay and mouse albumin specific ELISA, respectively. For *in-vivo* biodistribution studies, particles were loaded with Nile red, a hydrophobic fluorophore, and the relative fluorescence of tissues homogenized in DMSO was assessed. Acute liver failure was induced in mice by carbon tetrachloride injections, and protein loaded particles were delivered intravenously. Serum ALT levels were measured using ELISA, and histological changes in the liver were examined using routine staining.

**Results:** Characterization of alginate microparticles and several types of polyester nano- and microparticles revealed that modified PLGA/PHBV particles were the most efficient system for delivery of HGF, based on encapsulation efficiency and release dynamics. Total amount of the protein encapsulated was 180 and 60 ng per mg of particles, representing 51.7% and 14.7% theoretical encapsulation efficiency (all values are in reference to

PLGA/PHBV and alginate particles, respectively). Furthermore, alginate encapsulation resulted in release over 5 days whereas PLGA/PHBV released HGF over a 21 day period. Identically prepared particles were determined to efficiently encapsulate and release HIP/PAP. Primary mouse hepatocytes were successfully cultured in a 3-dimensional Matrigel culture system for as long as 7 days, as determined by cell viability studies, microscopy and hepatocyte-specific albumin secretion. Following the titration of effectively toxic concentrations of carbon tetrachloride, we investigated the ability of HGF and HIP/PAP to rescue hepatocytes from carbon tetrachloride induced toxicity in primary cell cultures. Several concentrations of growth factors were examined and a dose dependent ability of soluble growth factors to rescue primary hepatocytes from carbon tetrachloride toxic effects was demonstrated based on measures of cell viability, LDH release, as well as hepatocyte specific CYP3A4 P450 activity and albumin release. Released growth factors retained their bioactivity after encapsulation, determined by their ability to rescue hepatocytes when challenged by carbon tetrachloride. Several routes of administration were examined using fluorescently labeled polymeric particles, and the biodistribution in several organs based on the mode of administration was determined.

**Conclusions:** We hypothesized that controlled delivery of HGF and HIP/PAP using encapsulation in polymeric particles would provide a novel clinically relevant method to treat acute liver failure. We have successfully shown and optimized a delivery system capable of releasing biologically active hepatic growth factors and shown that they have protective effects in a primary hepatocyte culture model. Our *in-vivo* distribution data shows the feasibility of delivering polymeric particles to the liver. We are in the process of testing the system presented here in a mouse model of carbon tetrachloride induced liver failure. Our results to date suggest that controlled delivery of hepatic growth factors presents a useful potential approach as a treatment for acute liver failure or a bridge to liver transplant.