Controlled Delivery of Sonic Hedgehog Morphogen for Cardiac Repair

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Statement of Purpose: Approximately 1/3 of American adults have cardiovascular disease, the prevalence of which is expected to increase 25% by 2030. Current treatments only seek to maintain the reduced function of the damaged heart, presenting a clear need for regenerative therapies. Sonic Hedgehog (Shh) is a morphogen involved in heart development but only expressed in the post-natal heart following ischemic injury. Shh therapy may therefore temporarily recapitulate the embryonic signaling environment when the heart briefly has the ability to regenerate itself (Porrello ER. Science, 2011;331:1078-80). However, the clinical potential of Shh is limited by its short half-life in the body. We have developed a novel delivery vehicle for Shh consisting of heparin and a synthetic polycation which interact to form a coacervate. The objective of this work was to evaluate the potential of Shh coacervate to mitigate the damage caused by myocardial infarction in rats. To the best of our knowledge, this is the first investigation of sustained delivery of Shh for cardiac repair and regeneration.

Methods: The delivery vehicle was synthesized and prepared as previously described (Johnson NR. J Control Release, 2013;166(2),124-9). Shh was fluorescent-labeled and the Shh coacervate imaged by confocal microscopy. Loading efficiency and release of Shh from the coacervate in vitro was quantified by ELISA. Primary cardiac myocytes (CM) and fibroblasts (CFB) were isolated from 1-2d old SD rat pups. CMs pre-treated for 48h with Shh free or in the coacervate, or FGF2 as positive control, were exposed to 200μM H_2O_2 oxidative stress conditions and then analyzed using a caspase-3 apoptosis assay. Conditioned media from CFBs stimulated for 6, 12, 24, and 48h by Shh free, or delivered by the coacervate, was analyzed for levels of VEGF, SDF-1α, IGF-1, and Shh using ELISA and western blot. Myocardial infarction was induced by permanent LAD coronary ligation in 180-200g Lewis rats and saline, poly(ethylene glycol) (PEG) gel alone, Coacervate alone, or Gel+Coacervate was injected into the infarct. Relative amount of coacervate remaining in the heart wall 1d after injection was quantified by fluorescence microscopy. Cardiac function was assessed by echocardiography.

Results: Fluorescent imaging of the Shh coacervate showed spherical droplets of 0.5-10μm in size (Fig. 1a). The loading efficiency of Shh into the coacervate was 95% and release was slow and sustained over 21 days (Fig. 1b). Oxidative stress-induced CM apoptosis was reduced by pre-treatment with Shh coacervate compared to control while free Shh had no statistically significant effect (Fig. 1c). Shh coacervate was even superior to FGF2 which has been previously shown to protect CMs from oxidative stress. Shh coacervate also stimulated CFBs to upregulate secretion of Shh itself, and multiple trophic factors each with important roles in cardiac regeneration including VEGF for angiogenesis, SDF-1α for progenitor cell recruitment, and IGF-1 for cardioprotection (figure not shown). Shh coacervate was well-dispersed throughout the PEG gel (Fig. 1d) and the gel increased the retention of the coacervate in the heart wall 1d post-injection (Fig. 1e). Cardiac function was significantly higher in rats receiving injection of Gel+Coacervate at 2 and 4 weeks post-MI compared to injection of each alone (Fig. 1f).

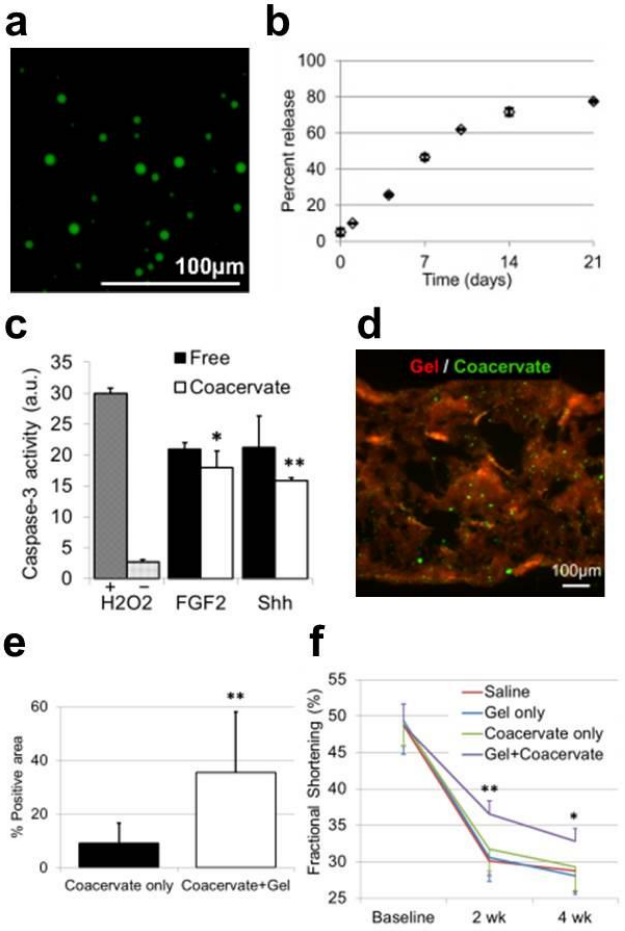

Figure 1. a) Shh coacervate fl. image. b) Shh release profile. c) CM apoptosis study. d) Shh coacervate in PEG gel fl. image. e) Coacervate retention 1d post-injection. f) Cardiac function post-MI. (*p<0.05, **p<0.01)

Conclusions: These results demonstrate that Shh coacervate can protect CMs from oxidative stress, upregulate secretion of multiple trophic factors by CFBs, and alleviate the damage caused by myocardial infarction.