Targeted Delivery of a TGF-β Receptor II Inhibitor Using Multifunctional Nanogels to Control Cardiac Fibrosis after Heart Failure

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Statement of Purpose: Heart Failure (HF) affects more than 6 million people in the US alone. The fatality rate is as high as 42.3% within 5 years of diagnosis. However, there lacks effective treatment to preserve heart function after HF[1]. Interstitial fibrosis is considered a key player in the progression of HF. After HF, transforming growth factor βs (TGFβs) are upregulated, leading to excessive collagen deposition. We found that a TGFβ receptor II binding peptide ECG can inhibit the TGFβ pathway, and in turn control cardiac fibrosis. In this study, we aimed to deliver ECG to the injured heart in a non-invasive and targeted manner. ECG was encapsulated in thermal- and pH- dual sensitive nanogels. Platelet membrane was cloaked on the nanogels to reduce cellular uptake by macrophage-like cells. A cardiac targeting peptide (CTP) APWHLSSQYSRT[2] was conjugated onto the platelet membrane surface to help the nanogels target the heart.

Methods: The platelet membrane cloaked nanogels (PMNG) were fabricated using the same method as our previous study[3]. MTT assay was performed on rat cardiac fibroblasts (RCFs) to study the cytotoxicity of ECG up to 100 μg/ml. To examine the effectiveness of ECG in maintaining fibroblasts phenotype, real-time RT-PCR and immunofluorescence (IF) staining were employed. RCFs were seeded on collagen-coated glass slides, and were treated with normal culture medium (Control group), 5 ng/ml TGFβ in culture media (T group), or 5ng/ml TGFβ and 50 μg/ml ECG in culture media (T+E group) for 24 hours. Fold change for mRNA expressions (α-SMA, CTGF, Fn1, and Col1A1) were calculated using the ΔΔCt method. β-actin was used as an internal reference. For IF staining, all cells were fixed and stained with α-SMA antibody. ECG was encapsulated in the nanogels during the fabrication. The loading concentration was 1 $mg_{ECG}/mg_{Nanogel}$. The ECG release study was performed by dialyzing ECG nanogel suspension against PBS for 28 days. The targeting ability of CTP to the heart was verified by In Vivo Imaging System (IVIS). Transaortic constriction (TAC) surgery was performed on female mice 8-10 weeks of age. PMNG were delivered via tail vein injection into the animals 14 days post-surgery, followed by echocardiography on days 7, 14, 21, and 28. The hearts were harvested 4 weeks after injection. The samples were fixed, embedded in paraffin, and transversely sectioned for hematoxylin and eosin (H&E), picrosirius red (PSR), and IF staining. *In vivo* Western Blot study was performed for markers α-SMA, total SMAD 2/3, phospho-SMAD 2/3.

Results: MTT assays showed that ECG had no significant cytotoxicity even when the concentration was as high as 100 μg/ml. ECG can effectively maintain the cardiac fibroblast phenotype under the presence of TGFβ1. At the mRNA level, the cells in the T+E group had significantly lower expressions of α-SMA, CTGF, Fn1, and Col1A1 compared to the T group. The administration of ECG in PMNG (PMNG/ECG group) significantly increased the ejection fraction (74% increase) and fractional shortening (81% increase) compared to the group treated with empty PMNG (PMNG group). ECG administration also reduced left ventricular diastolic/systolic volume and posterior wall thickness. In addition, total collagen in PMNG/ECG group was significantly lower than the PMNG group. Western blot study showed that α-SMA and phospho-SMAD 2/3 expressions in the PMNG/ECG group was downregulated.

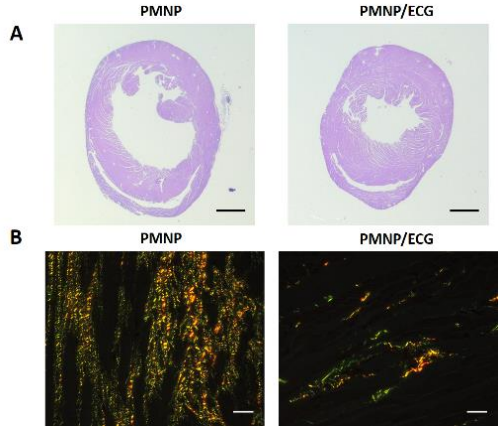

Figure.1 (A) Representative H&E staining images of heart sections (scale bar = 0.5 mm); (B) Representative PSR staining images of heart sections under polarized light microscopy (scale bar = 20 μm).

Conclusions: The TGF-β receptor II inhibitor peptide ECG was successfully delivered in thermal- and pH- dual sensitive nanogels with platelet membrane camouflage. ECG peptide showed great effectiveness in maintaining the phenotype of fibroblasts *in vitro* and *in vivo*, resulting in reduced cardiac fibrosis and improved heart function after HF.

References:

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