Interpenetrating Network Hydrogels for Enhanced Cardiac Retention of Extracellular Vesicles

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Statement of Purpose: The use of biomaterials to deliver extracellular vesicles (EVs) has emerged as a promising strategy in regenerative medicine to overcome the low retention rates of bolus EV injections. We and others have shown that controlled release of EVs from hydrogels aids in the recruitment of endogenous stem or immune cells and promotes tissue vascularization.[1] However, the therapeutic benefit of temporal control over EV release kinetics has not been investigated. To address this, we designed an injectable interpenetrating network (IPN) for the release of mesenchymal stromal cell derived (MSC)-EVs, building in a transglutaminase (mTGase) network into our already explored guest-host (GH) hydrogel for EV delivery. The secondary network offers enhanced mechanical and degradation properties to further investigate MSC-EV release. Further, we used a highly versatile metabolic labeling technique to label MSC-EVs for in vivo tracking. **Methods:** HA was modified with β- cyclodextrin (CD-HA) or adamantane (Ad-HA) and mixed to form GH hydrogels. IPN hydrogels were formed with the addition of gelatin (Gelita, 100 bloom; 5 wt%) cross-linked with mTGase (0.38 mg/mL) to GH network. For *in vivo* gel retention studies, Cy3 conjugated GH and IPN gels were injected into myocardium of healthy Wistar rats and explanted for Spectrum In Vivo Imaging Analysis (IVIS). For metabolic labeling, MSCs were cultured in methionine-depleted media containing 100 μM Azide-containing methionine analog. MSC-EVs were isolated using ultracentifugation, labeled using cycloaddition with DBCO-containing NearIR dye, and quantified for EV yield and labeling efficiency via Nanoparticle Tracking Analysis (NTA). For EV retention studies, metabolically labeled EVs were incorporated into PBS, GH, and IPN hydrogels, injected into myocardium, and explanted for IVIS. **Results:** We successfully formulated IPN hydrogels (Fig. 1A) that were injectable within 15 minutes after mixing and then underwent gelation with increased mechanical properties (Fig. 1B). IPN hydrogels were injected and retained within the rat myocardium for up to 14 days after injection, with increased retention over GH hydrogels alone as observed with varied IVIS images and quantification (Fig. 1C and D). MSC-EVs were labelled using a metabolic labelling technique with a NIR fluorophore with high efficiency (Fig. 2A, B). Labeled MSC-EVs were delivered to rat myocardium in saline or encapsulated in GH or IPN hydrogels. The EVs were cleared rapidly when injected with saline, without any noticeable signal after 1 day, whereas EVs were retained for 7 days when delivered in GH hydrogel and greater than 14 days in the IPN hydrogel (Fig. 2C, D). Ongoing work focuses on investigating the therapeutic benefit of extended MSC-EV delivery in a rat model of myocardial infarction.

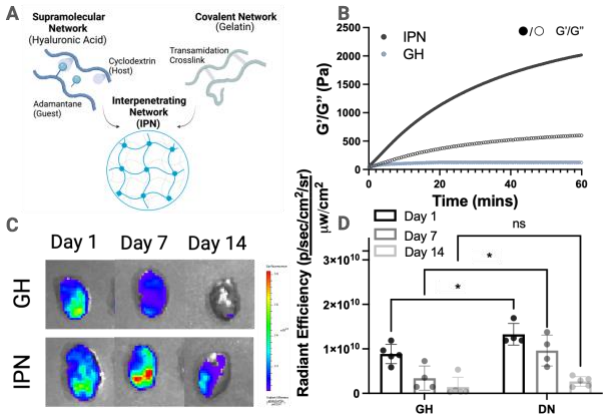

Figure 1: **A)** Schematic of IPN hydrogels from GH and gelatin-mTGase networks. **B)** Time sweeps (1 Hz, 0.5% strain) of GH and IPN hydrogels on rheometry. **C)** Representative IVIS images and **D)** quantification of Cy3-labeled GH and IPN hydrogels on Days 1, 7 and 14 after injection into the rat myocardium. * $p<0.05$

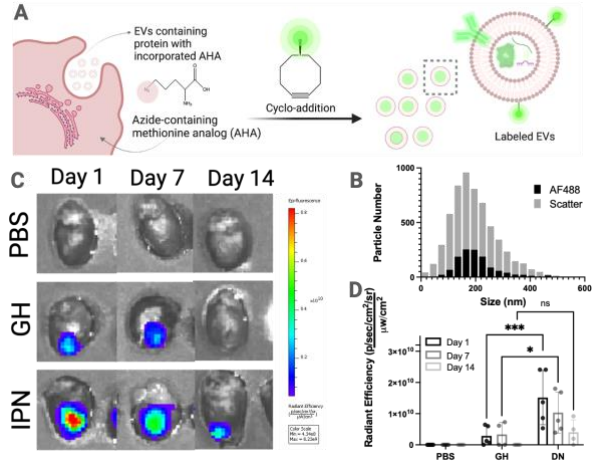

Figure 2: **A)** Schematic demonstrating the metabolic labeling of MSC-EVs. **B)** Characterization of EV particle distribution, and labeling efficiency on NTA. **C)** Representative IVIS Images and **D)** quantification of Cy3-labeled EVs in PBS, GH or IPN hydrogels on day 1, 7 and 14 after injection into the rat myocardium. * $p<0.05$

Conclusions: Towards improvement in promising EV therapies for cardiac repair, we sought to increase EV retention in the heart. This was accomplished through the design of an IPN hydrogel and imaging with metabolically-labelled EVs.

References: [1]Chen. *Cardiovasc. Res*. 2018; 114:1029-40.