Injectable acellular guest-host hydrogel for miR-302 delivery promotes cardiomyocyte proliferation

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Statement of Purpose: RNA-interference based technologies have great potential for cardiac therapeutics. We previously identified miR-302, a regulator of Hippo signaling, as inducing cardiomyocyte proliferation and rescuing cardiac function when administered systemically in a rodent model after myocardial infarction (MI).[1] To improve translation and reduce off-target effects, we sought to locally deliver miR-302 to the heart through an injectable hyaluronic acid (HA) hydrogel. Towards sustained release, cholesterol modified miR-302 mimics (miR-302-chol) were used to both promote transfection and introduce affinity for cyclodextrin-modified HA (CD-HA), which was then mixed with adamantane-modified HA (Ad-HA) to form injectable, shear-thinning hydrogels assembled through guest-host interactions.

Methods: *Material synthesis:* CD-HA and Ad-HA were synthesized as previously described.[2] *In vitro release and bioactivity:* CD-HA and Ad-HA were resuspended in miR-302 solution and mixed to form gels (5 wt%). Releasates were collected from gels over three weeks and added to neonatal mouse cardiomyocytes (NMCM) in culture, which were stained with Ki67, Troponin T, and DAPI. *In vivo bioactivity:* Gels were assembled as previously described and manually transferred to a 27G insulin syringe. A left thoracotomy was performed on C57BL/6 mice (7-9 weeks) to access the heart. For non-MI groups, injections (2 x 5 µL) were made inferolateral to the proximal LAD and hearts were explanted at five days for IHC. For MI, the LAD was ligated and injections (2 x 5 µL) were made in the border zone. At four weeks, cardiac function was analyzed by echocardiography and hearts were explanted for Mason's Trichrome staining.

Results:

Shear-thinning and self healing HA gels were formed through guest-host interactions of CD-HA and Ad-HA (Fig. 1A). miR-302-chol release was sustained over three weeks and release was attenuated when compared to unmodified miR-302 to confirm affinity to the gel (not shown). Releasates collected within this time period were added to NMCM, which led to increased proliferation from D0-D1 and D1-D4 compared to controls, confirming miR-302 release and bioactivity (Fig. 1B).

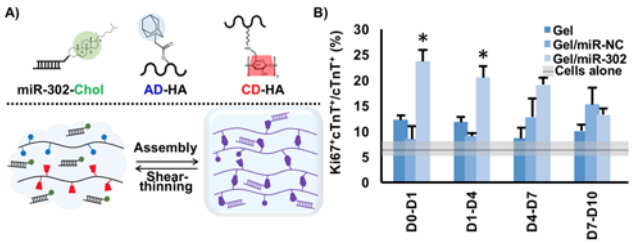

Figure 1. A) Schematic for guest-host mediated gel assembly (via CD-HA and Ad-HA interactions) and retention of miR-302-chol from the gel. B) Quantification of Ki67$^+$ NMCMs after exposure to gel releasates from different time intervals compared to empty gels and non-targeting miR controls (miR-NC). *p<0.05

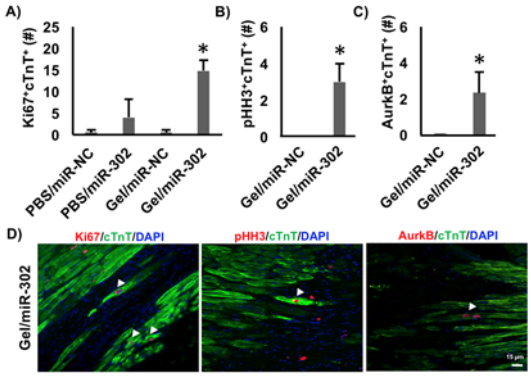

Figure 2. A) Ki67$^+$, B) phosphorylated Histone H3$^+$ (pHH3), and C) Aurora B Kinase$^+$ (AurkB) CM quantification per axial section five days after injection. D) Confocal images of proliferating CMs around injection site. *p<0.05

Gel/miR-302 or controls were injected into mouse myocardium without infarction. At five days, gel/miR-302 led to significantly increased Ki67$^+$ CMs surrounding injection sites compared to controls (Fig. 2A), suggesting proliferation *in vivo*. CMs were also positive for markers of mitosis, pHH3 (Fig. 2B) and AurkB (Fig. 2C). Representative IHC images are shown (Fig. 2D).

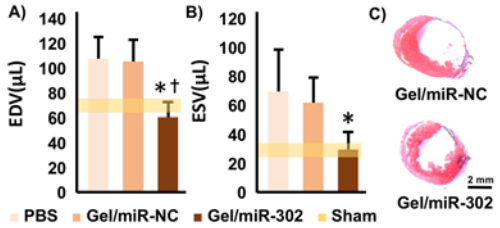

Figure 3. Echocardiographic measurements of A) end diastolic volume (EDV) and B) end systolic volume (ESV). *p<0.05 compared to PBS, †p<0.05 compared to gel/miR-NC. C) Mason's trichrome staining of axial sections.

In a mouse model of MI, gel/miR-302 significantly decreased EDV (Fig. 3A) and ESV (Fig. 3B) compared to controls at 4 weeks, indicating a reduction of ventricular dilation after MI. Mason's trichrome staining suggest gross improvements in these ventricular volumes and improved infarct and myocardial wall thickness (Fig. 3C). This suggests local, gel-mediated delivery of miR-302 limited pathologic remodeling.

Conclusions: We have developed an injectable, guest-host assembled miRNA delivery system that promotes cardiomyocyte proliferation and improves cardiac volumes from a single intervention. Ongoing studies are aimed at a mechanistic understanding of this process and optimizing this technology for large animal translation.

References:

1. Tian Y. Sci Transl Med. 7(279); 279ra38.
2. Rodell C. Biomacromolecules. 14(11);4125-34.