Utilizing Injectable Decellularized Extracellular Matrix Hydrogels for the Slow Release of MicroRNAs

Melissa J. Hernandez[1], Roberto Gaetani[1], Jessica L. Ungerleider[1], Joost P.G. Sluijter,[2] and Karen L. Christman[1]
[1]Sanford Consortium for Regenerative Medicine, Department of Bioengineering, University of California, San Diego, La Jolla, CA
[2]Department of Cardiology, University Medical Center, Utrecht, NL.

Statement of purpose: One main challenge for employing microRNAs (miRNAs) as therapeutics has been ineffective delivery. Upon injection, miRNAs rapidly diffuse from the injection site, and circulating RNases also quickly degrade these small oligonucleotides. Since previous studies have examined decellularized extracellular matrix (ECM) hydrogels for the delivery of growth factors[1,2], our objective was to evaluate these hydrogels for delivering miRNAs. We hypothesized that encapsulation of miRNAs within ECM hydrogels would prolong the release profile, allowing for greater therapeutic potential with *in vivo* applications.

Methods: Myocardial, skeletal, and lung ECM hydrogels were derived from porcine tissue and processed according to an established protocol[3]. Fabrication included decellularization with a detergent (sodium dodecyl sulfate) to remove cellular contents, lyophilization, milling, and a partial pepsin digestion to liquefy the ECM. Samples were then lyophilized again for long-term storage until being resuspended with sterile water immediately prior to use. Antimir and antagomir, miRNA inhibitors, were synthesized with the same 22-bp complimentary sequence, but the antagomir had a cholesterol group conjugated to the 3' end. Forty µg of Cy3-labeled antimir (n=3) or antagomir (n=3) were loaded in 200 µl of 6 mg/ml of each decellularized ECM hydrogel. All hydrogels were initially rinsed with 250 µl of 1X phosphate buffered saline (PBS) to remove any unincorporated antimir or antagomir. After this initial rinse, 250 µl of 1X PBS were added, and 200 µl of supernatant were collected every 24 ± 2 hours. Samples were incubated continuously at 37°C and placed on a shaker plate. Collagenase and 1.5M sodium chloride (NaCl) were added in place of 1X PBS at the end of the study to dissociate residual miRNA. Samples remained in the collagenase and 1.5M NaCl for 4 hours and 1 hour, respectively. Fluorescence measurements were utilized throughout the study to quantify miRNA release with a microplate reader. Values are graphed as mean ± SD. Samples from the release study were also evaluated for signs of degradation using a 20% polyacrylamide gel with 1X TBE running buffer. Gel electrophoresis was performed at 125V, and gels were stained with SybrSafe for imaging. Stock solutions of the antimir and antagomir, along with a 10 bp ladder were used for size comparisons.

Results: Although the antimir and antagomir shared the same oligonucleotide sequence, the presence of a cholesterol group on the antagomir largely affected the release profile. For the ECM hydrogels containing antimir, the majority of the miRNAs were released by day 8 (Figure 1). However, the antagomir was not fully released until the ECM hydrogels were degraded upon the addition of collagenase on day 15 (Figure 2).

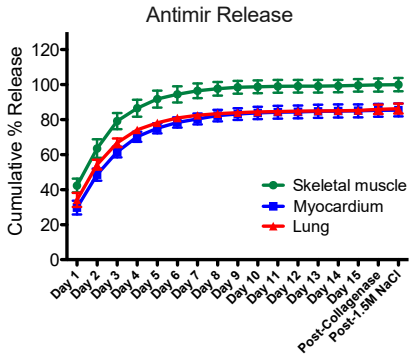

Figure 1. Release of antimir from ECM hydrogels.

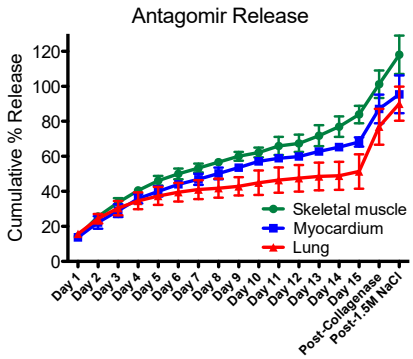

Figure 2. Release of antagomir from ECM hydrogels.

Antimir and antagomir samples also did not appear to be degraded upon being released from the ECM hydrogels. Imaging from gel electrophoresis revealed that the sizes of the antimir and antagomir samples from the release study were consistent with the stock solutions.

Conclusions: ECM hydrogels resulted in a steady release of the miRNAs over a two-week period *in vitro*. Slower release of the antagomir was likely due to electrostatic interactions between the attached cholesterol group and ECM hydrogel, as indicated by further release of the antagomir following incubation with 1.5M NaCl. In addition, the released antimir and antagomir did not appear to be degraded. This study establishes the ability of decellularized hydrogels to modulate the release of miRNAs, which could allow for increased efficacy by localizing the therapeutic benefits in the region of interest.

References: [1] Seif-Naraghi SB. Acta Biomater. 2012;8:3695-3703. [2] Sonnenberg, SB. Biomaterials. 2015; 45:56-63. [3] Ungerleider JL. Methods. 2015;84:53-59.