Grant Title: Design and Delivery of Advanced Therapeutics for the Treatment of Cerebrovascular Disease
Grant Number: MRM 2015 BB 004
Requester: Brenda Ogle PhD
Project Timeline: May 1, 2015 – April 30, 2016

Brief description of project:
New therapies that target the mechanism of aneurysm formation are in need. Delivery of mesenchymal stem cells (MSCs) to the site of an intracranial aneurysm could offer a less invasive means to stabilize or even repair an aneurysm prior to rupture as MSCs have been shown to limit inflammation, spur extracellular matrix production and even differentiate into endothelium and smooth muscle cells. To this end, we utilized an elastase-induced aneurysm model in canine carotid and cerebrovasculature. After formation of the aneurysm, MSCs were delivered to the site via custom catheter delivery. After treatment, time-of-flight magnetic resonance angiography (TOF MRA) was used to track the progression of the aneurysm. In addition, tissue sections at the aneurysm site and control tissues were probed for immune cell infiltration and extracellular matrix deposition. Quantitative analysis of staining outcomes suggests a decrease in inflammation in treated vs. control tissue. Our preliminary results support delivery of MSCs to the site of aneurysm as a minimally invasive means to stabilize an aneurysm and perhaps help avoid more invasive repair procedures.

Where did this project take place?
University of Minnesota Twin Cities
- Nils Hasselmo Hall
- Lions Research Building/Mcguire Translational Research Facility
- Experimental Surgical Services
- Center for Magnetic Resonance Research

People Impacted by project and where they are from:
The proposed treatment method has potential to impact those with diagnosed aneurysm in Minnesota and world-wide. As computed tomography (CT) scans and magnetic resonance imaging (MRI) become more common methods of disease detection, the inadvertent identification of cerebral aneurysms is on the rise. For example, people with frequent headaches are now undergoing these scans along with people who could have experience traumatic brain injury. Early detection is beneficial but comes with the complex and imperfect decision tree associated with a treatment plan. Plans could include watchful waiting, an open surgical procedure or endovascular repair. Regardless of approach, treatment remains dangerous with high in-hospital complication rates and long-term recurrence rates for both interventional procedures.

What was the outcome of the project? (Did the project work the way you expected it to? What were the successes? What were the failures? How did it impact regenerative medicine in Minnesota?

The primary successful outcome of the project was favorable support for the premise that MSCs delivered to the site of aneurysm might reduce inflammation associated with the site and thereby reduce the probability of future rupture. These results corresponded to the creation of an aneurysm in the carotid artery of dogs with subsequent delivery of canine MSCs via custom-made delivery catheter (Figure 1). Ballooning of the vessels in the right common carotid of the neck after treatment with elastase could be seen suggesting the breakdown of supporting structures in the vessel wall. TOF-MRA was used to detect and track progression of the carotid aneurysm over time. For better monitoring in future studies supplementing imaging with gadolinium, a magnetic resonance imaging (MRI) contrast agent, should provide better imaging capabilities. Aneurysms were created via exposure to elastase. To confirm elastin degradation, histologic sections from both MSC treated and untreated aneurysmal sites were stained using Movat's Pentachrome. Aneurysm sites were characterized by decided loss of tight elastin fibers (Figure 2). In addition, we stained histologic sections for CD90 to confirm MSCs were effectively delivered and maintained at the site of aneurysm. In fact, several cells were localized to the site even four weeks following cell delivery (Figure 3). Further, histological analysis of tissues from treated (MSC delivery) and untreated aneurysms showed a decrease in cellularity perhaps associated with immune suppression in the treated case (Figure 4). These data, plus results from two additional procedures currently underway will serve as strong preliminary data for larger extramural awards that garner sufficient funds to carry out conclusive studies in the dog model. Of important note, we have been unsuccessful in creating aneurysm in the cerebrovasculature. Early attempts yielded either no aneurysm or rupture. When these attempts failed, we utilized the carotid such that the therapeutic potential could be assessed. Thus we also intend to submit an NIH R21 proposal in response to a call for development of animal models such as these.

As noted above, if incoming data further support the concept of the award, the proposed treatment method has potential to improve outcomes for those with diagnosed aneurysm in Minnesota and world-wide.

Please list any of the following that have resulted from your Regenerative Medicine Minnesota grant funding:

Publications and/or manuscripts submitted for publication: N/A

Disclosures/patents: N/A

Other grant applications and/or awards: To be submitted following conclusion of the study: NIH R21, PAR-13-115, Improvement of Animal Models for Stem Cell-Based Regenerative Medicine; NIH STTR, PA-16-303, Omnibus Funding Opportunity

Responsible Spending:

All funds have been spent or encumbered according to the initial budget. Items include: canine specimens (4), surgical suite rental, animal per diems, MRI scan time, drugs (ferumoxytol and elastase), general medical supplies, ESS staff time, animal transportation.

Figure 1: Placement of the catheter around the aneurysm site. Balloons are inflated on either side of the aneurysm via the custom cell delivery catheter. Cells are injected into the area of restricted flow.

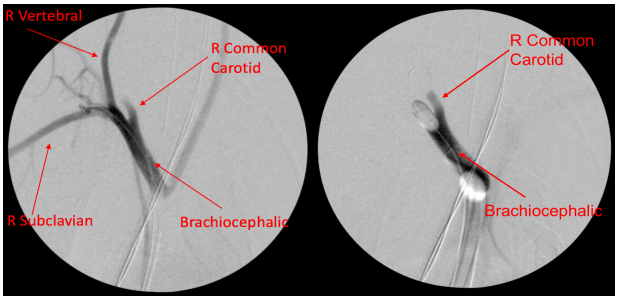
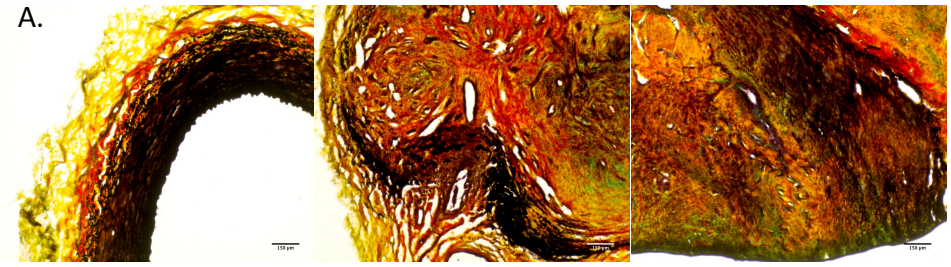


Figure 2. A. Movat's Pentachrome staining of control left carotid, the treated aneurysm, and the untreated aneurysm. In both treated and untreated putative aneurysm, elastin breakdown was noted, and both are filled with organized thrombus. Scale bar 150µm. **B.** Quantitative analysis of elastin breakdown.

A.



Left Carotid

Untreated

B.

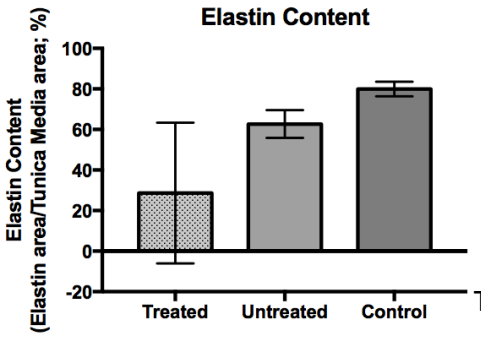


Figure 3: Immunohistochemistry of CD90 positive cells within the treated aneurysm, scale bar 25µm. Nuclei-DAPI (blue)

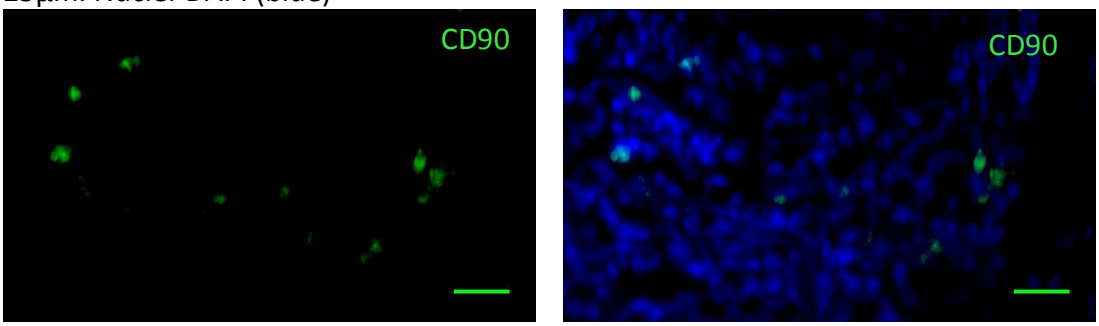
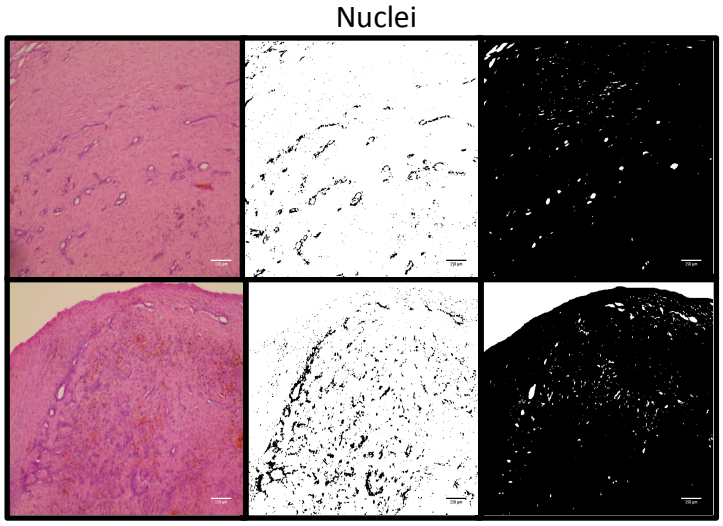


Figure 4: A. Hemotoxylin and eosin (H&E) staining of the treated and untreated vascular section of the aneurysms, split into nuclei and tissue area by Colour Deconvolution. Scale bar 150µm. **B.** Quantitative analysis of H&E; cellularity. Untreated aneurysm has greater cellularity suggestive of heightened immune response.

A.



B.