Minnesota Regenerative Medicine Progress Report
Due: July 31, 2018

Grant Title: Endothelialization of Engineered Coronary Artery Bypass Grafts Using Bone Marrow and Adipose-derived Mesenchymal Stem Cells

Grant Number: MRM 2015 1872

Principal Investigator: Robert T. Tranquillo, PhD

Project Timeline: July 1, 2015 - June 30, 2017 - NCE to June 30, 2018

Progress to Date:
For each aim, please describe progress and obstacles and/or achievements. Please address any deviations from estimated timelines and limit ***each*** response to 500 words. Figures and tables are allowed.

Aim 1: Assess the influence of physical stimulations (SS, CS, SS+CS) on retention, morphology, proliferation, and differentiation of MSC and ASC seeded sparsely or densely onto our TEVG (SS= shear stress, CS=cyclic stretching, MSC=mesenchymal stem cells, ASC= Adipose-derived stem cells)

Summary of Progress to Date:

We focused on ASC because their clinical advantages became apparent: they are available from most patients in sufficient number for coronary bypass graft seeding and may be effective as allogeneic cells.

Our studies of ASC exposed to SS have been published:
La, A. and R.T. Tranquillo, Shear Conditioning of Adipose Stem Cells for Reduced Platelet Binding to Engineered Vascular Grafts. Tissue Eng Part A, 2018. 24(15-16): p. 1242-1250.
A monolayer of cells was maintained up to 15 dyn/cm^2 constant SS and up to 15 dyn/cm^2 mean pulsatile SS for 6 days of shear flow. Platelet binding was reduced from 83% to 6% of surface area and nitric oxide production was increased 23-fold with 7.5-15 dyn/cm^2) constant SS, but not pulsatile SS, relative to cells cultured statically on the matrix for 6 days. The reduction in platelet binding varied from no reduction to maximum reduction over a constant shear range of approximately 2 to 4 dyn/cm^2, respectively. Collectively, the study supports the potential use of ASCs to seed the luminal surface of a vascular graft made from this biologically engineered matrix to confer an antithrombogenic surface during the development of an endothelium from the seeded cells or the surrounding blood and tissue.

Our studies of ASC exposed to CS and SS+CS have been completed and are reported in the Ph.D. thesis of A. La.

Aim 2: Assess the benefits of chemical pre-stimulation of MSC and ASC before exposure to physical stimulations.

Summary of Progress to Date:

In order to minimize the time needed to induce ASC differentiation and to maximize clinical relevance (for urgent CABG patients), it was decided to include growth factors in the medium during the SS, CS, and CS+SS, so this aim was not pursued.

Aim 3: Assess long-term in vivo hemocompatibility of MSC and ASC seeded sparsely versus densely following chemical and physical stimulations that are relevant to urgent CABG and result in

endothelial differentiation.

Summary of Progress to Date:

It was found that dense (monolayer) seeding was needed in order to retain cells under the SS used, so all studies were conducted with dense seeding. The motivation for sparse seeding is also irrelevant for ASC, which are normally of sufficient abundance in fat available from patients for seeding as a monolayer on a typical CAB graft.

The preliminary results imply that the reduced platelet binding observed in vitro with ASC-seeded grafts does not translate to reduced thrombogenicity in vivo and further study is needed to confirm and understand the outcome and to identify in vitro conditions that yield reduced thrombogenicity in vivo.

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

Publications and/or manuscripts submitted for
publication:
La, A. and R.T. Tranquillo, Shear Conditioning of Adipose Stem Cells for Reduced Platelet Binding to Engineered Vascular Grafts. Tissue Eng Part A, 2018. 24(15-16): p. 1242-1250.

La, A. and R.T. Tranquillo, Hemocompatible tissue-engineered vascular grafts using adult mesenchymal stem cells. Current Opinion in Biomedical Engineering 2018, 5:66–73

Disclosures/patents: n/a

Grant applications and/or awards:

Budget Update:
Please report the initial year's funding vs. spending and comment on any variance of >20% of estimated budget.

Overview:
Using lay language (in about 200 words), please describe your overall progress and how it is significant to the field of regenerative medicine and the Minnesota Regenerative Medicine vision, goals and objectives listed below.

These studies exploring the use of autologous adipose-derived stem cells as a potential surrogate endothelium for CAB grafts for emergent/urgent CABG patients reveal both the promise and complexity of this approach: the promising reduction in platelet binding to the CAB graft via flow-conditioning of seeded ASC observed in vitro did not reduce blood clotting in vivo, and may have even accentuated it relative to an unseeded graft. Further study is needed to confirm and understand this outcome, focusing on the fibrin formation aspect of clotting, and to identify in vitro conditions that yield reduced thrombogenicity in vivo. Discussions are ongoing with a foundation for further funding and/or support of this research for clinical translation.