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TITLE: “Transform Off-the-Shelf Synthetic Grafts to Autologous Conduits for Coronary Artery Bypass Grafting”

PRINCIPAL INVESTIGATOR: Yadong Wang

CONTRACTING ORGANIZATION: Cornell University, Ithaca

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14. **ABSTRACT**

**INTRODUCTION: Coronary arterial disease is the leading cause of death in the United States in both women and men. Arterial disease of the heart alone costs well over $2.5B for the VA system alone. To meet this challenge, we design vascular grafts made of special rubbers that are designed and patented specifically for vascular grafting. Unlike current grafts on the market or in clinical trials, the patients' own body will change these grafts over time to their own vessels. The two aims of this project are: 1. Finalize graft fabrication methodology and select the best design using a rat carotid artery model. And 2. Evaluate the performance of the selected grafts in a pig coronary artery bypass grafting model.**

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REPORT: PR181709 - “Transform Off-the-Shelf Synthetic Grafts to Autologous Conduits for Coronary Artery Bypass Grafting”

INTRODUCTION: Coronary arterial disease is the leading cause of death in the United States in both women and men. Arterial disease of the heart alone costs well over $2.5B for the VA system alone. To meet this challenge, we design vascular grafts made of special rubbers that are designed and patented specifically for vascular grafting. Unlike current grafts on the market or in clinical trials, the patients’ own body will change these grafts over time to their own vessels. The two aims of this project are: 1. Finalize graft fabrication methodology and select the best design using a rat carotid artery model. And 2. Evaluate the performance of the selected grafts in a pig coronary artery bypass grafting model.

KEYWORDS: Biodegradable synthetic vascular grafts, off-the shelf availability, host remodeling

ACCOMPLISHMENTS:

What were the major goals of the project?

| **Specific Aim 1:** Finalize graft fabrication methodology and select the best design using a rat carotid artery model | **Timeline** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) | **Completion date** |
|---|---|---|---|---|
| **Major Task 1:** Manufacturing of PGS-PCL and PGS-PDO grafts | Months | | | |
| Subtask 1: Produce grafts with thin and thick PCL fibers in sheath<br>• Already produced and published how grafts with PCL fibers perform in the preliminary study<br>• Thin PCL fiber grafts will be made for proteomic and RNAseq analysis | 1-2 | Wang | | 9-2019 |
| Subtask 2: Characterize grafts with thick PCL fibers | 2 | Wang | | 10-2019 |
| Subtask 3: Produce grafts with thin PDO fibers in sheath | 2-3 | Wang | | 10-2019 |
| Subtask 4: Characterize grafts with thin PDO fibers | 3 | Wang | | 11-2019 |
| Subtask 5: Produce grafts with thick PDO fibers in sheath | 3-4 | Wang | | 11-2019 |
| Subtask 6: Characterize grafts with thick PDO fibers | 4 | Wang | | 01-2020 |

| | | | | |
|---|---|---|---|---|
| **Major Task 2:** Testing of PGS-PCL and PGS-PDO grafts in a carotid artery model | | | | |
| Subtask 1: Implant grafts with thick PCL fibers in sheath | 3 | Wang | | 10-2019 |
| Subtask 2: Examine host response to grafts with thick PCL fibers in sheath | 3-7 | Wang | | 02-2020 |
| Subtask 3: Implant grafts with thin PDO fibers | 4 | Wang | | 01-2020 |
| Subtask 4: Examine host response to grafts with thin PDO fibers in sheath | 4-8 | Wang | | 04-2020 |
| Subtask 5: Implant grafts with thick PDO fibers | 5 | Wang | | 02-2020 |
| Subtask 6: Examine host response to grafts with thick PDO fibers in sheath | 5-9 | Wang | | 06-2020 |
| Subtask 7: Analyze explants, examine data and select best design | 9-11 | Wang | Green | 08-2020 |
| Subtask 8: Repeat the test of the best design in rats | 11-14 | Wang | | Ongoing* |
| Subtask 9: Analyze the 2nd test and confirm the performance of the selected graft | 12-14 | Wang | Green | Delayed* |
| Subtask 10: Conduct autologous vein CABG study | 1-12 | | Green | Delayed* |
| Subtask 11: Prepare manuscript and presentations of the rat study | 14-18 | | | Ongoing* |
| **Specific Aim 2:** Evaluate the performance of the selected grafts in a porcine CABG model | **Timeline** | **Site 1** (Initiating PI) | **Site 2** (Partnering PI) | |
| **Major Task 1:** Manufacturing of synthetic grafts for pig study | Months | | | |
| Subtask 1: Scale up the best design for porcine grafts | 14-16 | Wang | | |
| Subtask 2: Produce sufficient amount of grafts for the porcine study | 17-36 | Wang | | |
| Subtask 3: Characterize and quality control of the porcine grafts | 14-36 | Wang | | |
| **Major Task 2:** Porcine CABG study | Months | | | |
| Subtask 1: Establish the baseline performance of vein grafts in CABG | 13-16 | | Green | |

| | | | | |
|---|---|---|---|---|
| Subtask 2: Implant synthetic grafts from Subtask 2 in porcine CABG model. | 15-36 | | Green | |
| Subtask 3: Postoperative follow-ups, flow performance, fluoroscopy and animal care | 16-36 | | Green | |
| Subtask 4: Perform histology study | 14-36 | | Green | |
| Subtask 5: Prepare manuscript and presentations of the pig study | 30-36 | Wang | Green | |
| Subtask 6: Prepare continuation patent application(s) if warranted (patent already issued for basic graft design) | 24-36 | Wang | Green | |

**Accomplished under these goals:**

14 of the 17 subtasks for year 1 was accomplished. Two were delayed because we were allowed to continue the in vivo studies already initiated by mid-March when Cornell was shut down. But we were not allowed to order any new animals or start any new experiments from mid-March till early June. Upstate Medical were shut down and delayed the autologous vein grafting study, but we were able to get the ACURO protocol approved and will start the experiment soon. One subtask was accomplished early: We used the lab shutdown time to write the manuscript and was able to start it early.

The major activities and specific objectives are summarized in the above SOW table. I summarize the significant results and key outcomes below:

**Graft design**

We have tested the impact of fiber diameters and polymer types have on host remodeling. We discovered that PDO is not as compatible as PCL. So the sheath material will be PCL from now on. We learned that larger diameter fibers induce more healing response and lower inflammation. We have successfully improved the batch consistency of our graft fabrication process. The next step is to scale up the graft for porcine study.

**Genomics**

Single-cell RNA sequencing (scRNA-seq) is a powerful approach to study the vascular graft remodelling response because it reveals the transcriptional state of vascular grafts with single-cell resolution. We generated scRNA-seq libraries, using the 10X Genomics Single Cell Gene Expression v3 platform, for 3 samples (graft, distal, proximal) at 3 months post implantation using a 20% PCL graft. We also generated libraries for 4 samples (graft, distal, proximal, native)

at 6 months post implantation using 14% PCL graft. A control vessel library of a non-implanted rat was also collected generating 8 samples for scRNA-seq analysis. The single-cell transcriptomes of 24,882 total cells were uncovered across 8 samples after QC filtering. We identified 8 transcriptionally distinct cell types including 2 types of smooth muscle cells, 2 types of endothelial cells, 2 types of macrophages, T cells, and fibroblasts. Comparing the relative cell type composition across the samples, we find that graft samples at 3 and 6 months post implantation contained a higher percentage of macrophages and lower amount of smooth muscle cells relative to the control vessels. We also find that B and T cells in the graft at 3 months post implantation were more active in remodelling the graft than the same cell types in the graft at 6 months after implantation. Thus, scRNA-seq revealed cell-type specific responses during different stages of vascular graft remodelling that would be missing in standard tissue level RNA sequencing.

## Proteomics

### Protein extraction

2 mg rat vessel tissues were cut into cubic pieces and resuspended in 50 µl lysis buffer consisting of PBS (pH 7.4), 6M Urea, 2M Thiourea, 1% SDS and 50mM dithiothreitol (DTT) (ref#1). Then the samples were placed onto a vortex mixer (Fisher) with 1800 rpm at room temperature for 30 min., followed by sonicated for 1 h. After that, samples were centrifuged at 16,000g for 10 minutes, soluble fraction was transferred into a clean Eppendorf tube with leaving remaining insoluble pellet in the sample tube. Repeat the extraction steps and pool the extracted proteins together. Protein quantitation was done using a Bradford assay, then reduced by 10 mM DTT at 34 °C for one hour and alkylated with 50 mM iodoacetamide (IDA) inhibiting light at room temperature for 45 min, followed by quenched with a final concentration of 52 mM DTT. Phosphoric acid was added to each sample to a final concentration of 0.9% and then precipitated with 6-fold (v/v) binding buffer containing 0.1 M tetraethylammonium bromide (TEAB) and 90% methanol, pH 7.1. The precipitated proteins were loaded into S-Trap micro cartridges (Protifi), and washed for three times with 150 µL binding buffer by centrifuging at 4000 g for 30 seconds. Trypsin (2 µg) in 50 mM TEAB was added to each of the samples and incubated at 37 °C overnight for digestion. Tryptic peptides were eluted with 40 µL 50 mM TEAB, 0.2% formic acid and 50 % acetonitrile in 0.2% formic acid, then all eluents were pooled together.

To the remaining insoluble pellet, add 4 µg trypsin in 40 µl 50 mM TEAB and incubated in 37 °C overnight for digestion. After that, centrifuge at 16000 g for 10 min and transfer the supernatant to a clean tube, add 10 mM DTT for reduction at 34 °C for one hour and 50 mM IDA for alkylation at room temperature for 45 min, followed by quench with 25 mM DTT. Rinsed sample tube with an additional 40 µl 50 mM TEAB, and combine with the supernatant. Pooled the pellets digested peptides and the s-trap digested peptides together and lyophilized.

Prior LC-MS/MS analysis, MCX cartridges (Waters) was utilized for cleaning samples by the following steps: the cartridges were conditioned with 2 × 1mL methanol, 1 mL 10 mM HCL and equilibrated with 2 × 1mL 10 mM $KH_2PO4$ in 5% acetonitrile (ACN) (pH 3.0). The digested samples were in 1 mL 10 mM mM $KH_2PO4$ in 5% ACN (pH 3.0) and applied slowly through cartridges. Washed first with 2 × 1 mL of equilibration solution, followed by a second washed with 1 ml 95% ACN in 5 mM HCL, then 1 mL 10%ACN. Peptides were eluted with 2 × 0.6 mL of 75% ACN in 10% $NH_4OH$. Immediately after elution, Each sample was spiked with tryptic digests of ovalbumin (1.5 pmol) used as an internal standard for subsequence nanoLC-MS/MS data acquisition. A final 50 fmol of ovalbumin digests were injected in each sample.

All samples were filtered with 0.22 µm spin filter (Costar) and dried to dryness in the speed vacuum.

**Protein Identification and quantification by nano LC/MS/MS**

The tryptic digested peptides for each of the samples were reconstituted in 120 µL of 0.5% FA in 2% Acetonitrile (ACN). NanoLC-MS/MS analysis was carried out using an Orbitrap Fusion™ Tribrid™ (Thermo-Fisher Scientific, San Jose, CA) mass spectrometer equipped with a nanospray Flex Ion Source, and coupled with a Dionex UltiMate3000RSLCnano system (Thermo, Sunnyvale, CA) (ref#2,#3). The extracted peptide samples (4 µL) were injected onto a PepMap C-18 RP nano trapping column (5 µm, 100 µm i.d x 20 mm) at 10 µL/min flow rate for rapid sample loading and then separated on a PepMap C-18 RP nano column (2 µm, 75 µm x 25 cm) at 35 °C. The tryptic peptides were eluted in a 90 min gradient of 5% to 30% acetonitrile (ACN) in 0.1% formic acid at 300 nL/min., followed by an 8 min ramping to 90% ACN-0.1% FA and an 8 min hold at 90% ACN-0.1% FA. The column was re-equilibrated with 0.1% FA for 25 min prior to the next run. The Orbitrap Fusion is operated in positive ion mode with spray voltage set at 1.6 kV and source temperature at 275°C. External calibration for FT, IT and quadrupole mass analyzers was

performed. In data-dependent acquisition (DDA) analysis, the instrument was operated using FT mass analyzer in MS scan to select precursor ions followed by 3 second "Top Speed" data-dependent CID ion trap MS/MS scans at 1.6 m/z quadrupole isolation for precursor peptides with multiple charged ions above a threshold ion count of 10,000 and normalized collision energy of 30%. MS survey scans at a resolving power of 120,000 (FWHM at m/z 200), for the mass range of m/z 375-1575. Dynamic exclusion parameters were set at 50 s of exclusion duration with ±10 ppm exclusion mass width. All data were acquired under Xcalibur 3.0 operation software (Thermo-Fisher Scientific).

**Data analysis:**

The DDA raw files for CID MS/MS were subjected to database searches using Proteome Discoverer (PD) 2.3 software (Thermo Fisher Scientific, Bremen, Germany) with the Sequest HT algorithm. Processing workflow for precursor-based quantification. The PD 2.3 processing workflow containing an additional node of Minora Feature Detector for precursor ion-based quantification was used for protein identification and protein relatively quantitation analysis between samples. The database search was conducted against Ruttus NCBI fasta database that has 46182 entries downloaded plus an additional sequence of ovalbumin isoform X1. Two-missed trypsin cleavage sites were allowed. The peptide precursor tolerance was set to 10 ppm and fragment ion tolerance was set to 0.6 Da. Variable modifications of methionine oxidation, deamidation of asparagines/glutamine, acetylation of protein N-terminus, and fixed modification of cysteine carbamidomethylation, were set for the database search. Only high confidence peptides defined by Sequest HT with a 1% FDR by Percolator were considered for the peptide identification. The final protein IDs contained protein groups that were filtered with at least 2 peptides per protein.

Relative quantitation of identified proteins between the control and graft/proximal/distal samples was determined by the Precursor-based Label Free Quantitation (LFQ) workflow in PD 2.3. The precursor abundance intensity for each peptide identified by MS/MS in each sample were automatically determined and their unique peptides for each protein in each sample were summed and used for calculating the protein abundance by PD 2.3 software with normalization set to ovalbumin isoform X1. In each sample, ovalbumin isoform X1 was identified with more than 50% sequence coverage and >10 unique peptides. Protein ratios were calculated based on pairwise ratio for graft/proximal/distal sample versus control sample.

Training and professional development opportunities the project provided

The project was not intended to provide training and professional development opportunities. However, the staff, students and postdocs working on the project learned new analytical skills in material fabrication, genomics, and proteomics. They were also trained in professional writing while reporting the discoveries in the manuscript.

How were the results disseminated to communities of interest?

Manuscript under preparation. Intended to submit to Advanced Functional Materials.

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What do you plan to do during the next reporting period to accomplish the goals?

We will finish the rat carotid selection step, scale up the selected graft design for the pig study, finish pig autologous vein implantation, and begin implanting the synthetic grafts in year 2. We may be able to bring our progress back on track if the pandemic doesn't persist.

IMPACT:

The impact of what we accomplished so far is the material choice of vascular graft. We learned that PCL is a better sheath material than PDO. We learned that thicker fibers induce more favorable host response. These have broad impact on host-materials interface beyond vascular graft field. The fabrication method developed in this project will also be useful for materials science in general.

We expect this technology will be ready for transfer upon completion of the pig study. The broad impact will be realized for the healthcare industry after a successful commercialization, which present its own challenger beyond the scope of the current grant.

What was the impact on society beyond science and technology?

Nothing to Report.

Describe how results from the project made an impact, or are likely to make an impact, beyond the bounds of science, engineering, and the academic world on areas such as:

Nothing to Report.

CHANGES/PROBLEMS:

Changes in approach and reasons for change

Nothing to Report

Significant changes in use or care of human subjects

Nothing to Report

Significant changes in use or care of vertebrate animals.

Nothing to Report

Significant changes in use of biohazards and/or select agents

Nothing to Report

PRODUCTS:

Publications, conference papers, and presentations

Nothing to Report.

Technologies or techniques

Identify technologies or techniques that resulted from the research activities. In addition to a description of the technologies or techniques, describe how they will be shared.

Nothing to Report.

Other Products

Nothing to Report.

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Provide the following information for: (1) PDs/PIs; and (2) each person who has worked at least one person month per year on the project during the reporting period, regardless of the source of compensation (a person month equals approximately 160 hours of effort). If information is unchanged from a previous submission, provide the name only and indicate "no change."

Example:

Name: **G. Randall Green**

Project Role: **PI**

Researcher Identifier (e.g. ORCID ID): **https://orcid.org/0000-0002-9419-0794**

Nearest person month worked: **1.8**

Contribution to Project: **Dr. Green has written a protocol that was approved by the SUNY Upstate Institutional Animal Care and Use Committee(IACUC) as well as the Animal Care and Use Office (ACURO).**

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

**Nothing to Report.**

If the active support has changed for the PD/PI(s) or senior/key personnel, then describe what the change has been. Changes may occur, for example, if a previously active grant has closed and/or if a previously pending grant is now active.

**Nothing to Report.**

What other organizations were involved as partners? **No other institution, Upstate Medical University is the only partner.**

Describe partner organizations - academic institutions, other nonprofits, industrial or commercial firms, state or local governments, schools or school systems, or other organizations (foreign or domestic) - that were involved with the project. Partner organizations may have provided financial or in-kind support, supplied facilities or equipment, collaborated in the research, exchanged personnel, or otherwise contributed.

**Nothing to Report.**