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TITLE: Raman Spectroscopy and 3D Imaging as Decision Support Tools in the Assessment of Neuronal Fibrosis and Sarcopenia in Veterans and Combat Casualty Amputees

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14. ABSTRACT
Purpose: To identify Veteran-centric predictors of wound healing and ambulation after major amputation.
Scope: Utilize clinical data, imaging, tissue, and surveys to determine important causes.
Major Findings: Nerve fibrosis correlates with major amputation. Wound healing is slow.
Results: Veteran population with severe disturbances in pain and quality of life metrics.
Significance: Nerve fibrosis is important research topic in limb salvage, and Veterans undergoing major amputation may benefit from individualized healing and rehab strategies.

15. SUBJECT TERMS
Amputation; Nerve Degeneration; Sarcopenia; Raman spectroscopy

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1. Introduction

Veterans and combat casualties are at an increased risk of major amputation compared to the civilian population. Major amputations are extremely morbid but medically necessary procedures that are designed for the patient to ambulate after their wound heals. While many combat casualties and some veterans are able to ambulate after major amputation, these successes have not substantially improved in recent years. *The reason for this discrepancy in ambulation status after major amputation in veterans, despite exciting advances in prosthetic science, is not clear and relatively understudied. The purpose of this project is to identify the role that the quantity and quality of remaining skeletal muscle and degree of neuronal fibrosis play in identifying Veterans that are "at risk" of poor prosthetic fit and not ambulating after major amputation. This work will test the hypothesis that sarcopenia, ischemic myopathy, and neuronal fibrosis contribute to impaired prosthetic fitting and subsequent likelihood of ambulation in veterans after major amputation.* Here we collected and analyzed patient specific data to quantify how our patients' loss of skeletal muscle mass (sarcopenia), degree of ischemic myopathy, and degree of neuronal fibrosis impacts prosthetic fit and subsequent ambulation after major amputation.

The scope of this project included collecting patient specific information from Veterans who underwent major amputation. This data was then be used to create a predictive model for "at risk" veterans.

This proposal addressed the need outlined in the 2014 Orthotics and Prosthetics Outcomes Research Award (OPORA) for: patient-centric outcome assessments; generate new knowledge that can be developed in new clinical practice guidelines and algorithms for use of prosthetic and orthotic devices; and will develop important biologic data about amputated limbs that have the potential to develop improved prosthetic devices.

2. Keywords
Amputation; Nerve Degeneration; Sarcopenia; Raman spectroscopy

3. Accomplishments

Major goals of the project:

IRB approval AVAMC: Target date December 2015; Completed on time
IRB approval WRMC: Target date December 2015; not accomplished

Specific Aim 1: To build a predictive model for Veterans and Service members that segregates those who are able to walk after major amputation from those who do not walk.
Task:

a. Anticipated enrollment of approximately 51 patients
   Target September 2016; 58% enrollment; Data Completed June 2018 (30/51 enrolled)
b. Clinical data collection (including nerve and muscle analysis) as outlined below (Table 1)

   Target November 2016; 58% enrollment; Data Completed June 2018 (30/51 enrolled)

c. Raman spectroscopic assessment of tissue level compositional changes
   Target September 2016; Data completed September 2018.
d. Exploratory model building; Data completed 12/14/2018.

Table 1. Patient data to be collected.

Clinical Data Collection Time Points	Preoperative	Postoperative	1 month postop	3 month postop	6 month postop
Level of Amputation		X			
Wound Healing		X			
Time to Prosthetic Fit			X		
Time to Ambulation			X		
Muscle volume metrics	X		X	X	X
Brief Pain	X		X	X	X

Inventory					
Prosthetic Fit Comfort Score	X		X	X	X
Hauser Ambulation Index	X		X	X	X

Specific Aim 2: To prospectively test the ability of the model developed in Specific Aim 1 to predict future ambulatory status in our Veteran and combat casualties undergoing major amputation.

Task:

a. Anticipated enrollment of approximately 51 new patients in year 2
b. Validate our model developed in Specific Aim 1 using this new cohort and collected parameters as described in Specific Aim 1.

Initial Target completion: September 2017. Due to slower than expected enrollment, the model was built but validation utilized existing data and not additional patients.

Actual completion was December 2018.

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Major Accomplishments:

Major activities:

Methods: Veterans who had decided to pursue major amputation were recruited into this IRB approved prospective study.

30 Veterans were enrolled and evaluated in this project. 26 below knee amputations (BKA) and 4 above knee amputations (AKA). We utilized cross sectional imaging, survey data, clinical outcomes including time to healing, tissue from amputated specimens, whether or not Veteran received a prosthetic, and whether the Veteran walked with their prosthesis. We then used this data to build and back validate a model for predicting both desired and undesirable outcomes. As such this comprehensive evaluation of Veteran amputees has identified a number of important results and findings.

Specific Objectives:

1. Utilize cross-sectional imaging of muscle to correlate with amputation healing.
2. Survey Data for quality of life and pain score. Veterans participated in SF36, Brief Pain Inventory (BPI), and Hauser ambulation index. Length of stay (LOS), time until healing, re-operation, fate of contralateral limb, and time to prosthesis, and ambulation status were tracked. Preoperative CT imaging was utilized to evaluate muscle mass of the sartorius and gastrocnemius, which were normalized as ratios to the non-amputated limb. Veterans have been segregated

into normal (<2 months) and delayed (>2 months) wound healing. Data was analyzed by unpaired student t-test or Fisher's exact test as appropriate in GraphPad Prism and statistical significant difference was considered at $p<0.05$.

3. Clinical outcomes including time to healing.
4. Tissue analyses.

Biopsied Tissue. Twenty-six peripheral nerve biopsies collected from 24 patients were obtained from discarded limbs following amputation due to underlying pathology. Eleven peripheral nerve biospies were collected from 11 cadaver subjects following death of unrelated pathology. These cadaver subjects were selected to be free of underlying pathology related to peripheral arterial disease and serve as negative control. Each specimen was divided into two portions: approximately 1 inch of the proximal portion was snap frozen (-80 °C) for collagen assay and a remaining distal 2 inches cm was reserved for Raman spectroscopic analysis.

Raman Spectroscopy

Nerve biopsies for were examined *ex vivo* via Raman spectroscopy using a Kaiser Rxn1 PhAT probe 785 nm system (Kaiser Optical Systems, Inc., Ann Arbor, MI) with 3 mm diameter excitation spot size. Dark subtracted and intensity-corrected spectra were be acquired at six locations within each. Preprocessing of spectra was conducted using in-house MATLAB® scripts. Spectra was truncated to 600-1800 cm^{-1} and baseline subtracted using a fourth-order polynomial fitting routine described in Cao and Freeman [1]. Finally, spectra were intensity normalized to the phenylalanine band at 1004 cm^{-1}. Following preprocessing, spectra were curve fit using mixed Gaussian and Lorentzian functions of known Raman spectral bands based on the algorithms of GRAMS/AI software fitting features (Thermo Fisher Scientific, Madison, WI). Band area ratios (BARs) were calculated using curve fit derived band areas of peaks of interest. BARs calculated include sphingomyelin content [(719+760)/1004 cm^{-1}]; cholesterol content [(608+700)/1004 cm^{-1}]; protein-lipid disorder (1240/1270 cm^{-1}), a measure of β-sheet and disordered protein over α-helix, ordered protein and lipids; and protein disorder I (1240/1340 cm^{-1}), a measure of β-sheet and disordered protein over total protein.

5. Model development

Model development for prediction of future ambulation

Univariate analysis of the data set in year 1 provides basic descriptive statistics. Since ambulation potential depends on multiple factors, multivariate modeling was employed.

Regression Analysis Relating Raman Measurements to Intraneural Damage

Introduction: Regression analysis was performed to determine if Raman measurements could be used to reliably estimate intraneural damage. The objective of the analysis was two-fold. First, if a regression model could be constructed then it is anticipated that follow-on work could elucidate the fundamental chemistry related to intraneural damage. Additionally, positive results would be a step towards a methodology for fast, objective and inexpensive measurements of intraneural damage. The regression method employed a partial least squares model and leave-one-subject-out cross-validation (CV). CV was used to mimic and emphasize the regression model's *predictive* capability

in contrast to a less informative model *fit* criterion. The results of the analysis were positive however due to the small data set analyzed, further evidence is required to reproduce and validate the results. Results may be strengthened by including complete and appropriate clinical markers within the model.

Introduction to Regression Analysis. Regression analysis is a mathematical tool for relating measurements on one system to another.[a] Regression can be described for a simple case as follows. Imagine measuring the Raman signal from nerve tissue at a single frequency x and obtaining the corresponding intraneural damage score y. The objective of linear regression analysis is to find a "regression coefficient" b that relates x to y via the relation $y = xb$ in general. Finding this relationship can help understand the fundamental chemistry related to intraneural damage, and provide a fast, objective and inexpensive method for measuring it. Although finding b is easy for a single set of measurements x and y, this estimate isn't typically statistically relevant (generally useful) and instead it is best to make the estimate based on many measurements from neural tissue from many subjects. Therefore regression analysis focuses on finding the "best" b for a set of measurements on M subjects: $x_m b = y_m + e_m$ for $m = 1,...,M$. In this equation e_m is an error term that is minimized over the M measurements to find the "best" b. For example, the most common estimator minimizes the sum of squared errors to provide a least squares estimate $\hat{b}$ where the $\hat{}$ indicates an estimated quantity. Unfortunately, there are many chemical and physical effects that interfere with the Raman measurement of neural tissue and these interferences inhibit the ability to obtain a good estimate of intraneural damage from measurements at a single Raman frequency. Fortunately, Raman measurements are made at multiple frequencies spanning a wide spectrum of signals that can be used to characterize this "clutter" signal and that it may be possible to provide much better regression models using multivariate regression analysis.[b]

Raman spectra are typically measured at a range of N frequencies and the corresponding linear multivariate model is given by $x_{m,1}b_1 + x_{m,2}b_2 + ... + x_{m,N}b_N = y_m + e_m$. Instead of estimating one coefficient now N coefficients must be estimated. In this example, the collection of Raman measurements is considered the X-block or predictor block and the intraneural scores are considered the Y-block or predictand block. Due to mathematical reasons some practitioners try to reduce this number of coefficients to estimate by selecting key frequencies.[1] However, modern mathematical tools such as partial least squares (PLS) [b,c] and software tools [d] enable regression analysis utilizing many (hundreds or thousands) spectral frequencies. In addition to PLS, there are several methods that can be used to estimate the regression the coefficients (a.k.a., perform model identification). However, to meet the stated objective it is critical that the estimated model be generally applicable to neural tissue from many subjects and in this

[1] These problems are due rank deficiency or ill-conditioning in the measurements due to redundancies (or correlation) between frequencies. Selection of key Raman frequencies via variable selection can be useful but it reduces the ability to take advantage of signal averaging and noise filtering (a.k.a., the "multivariate advantage") when using multiple spectral frequencies.

work cross-validation was used to estimate the general predictive ability of the estimated regression model.[e]

Regression model *fit* statistics are based on a model estimated using all M samples. One statistic often reported is the root-mean-squared-error-of-calibration (RMSEC). RMSEC is the square root of the mean of e_m^2 over all M measurements. For example, for the simple "one frequency model," RMSEC can be interpreted as the standard deviation of $y - x\hat{b}$ and it is a measure of how well y is *fit* by $x\hat{b}$. In contrast to measuring a model's fit, cross-validation (CV) is a process that attempts to mimic how the regression model might perform on new measurements – it is a test of the model's *predictive* capability and general usefulness. For CV, the corresponding statistic reported is the root-mean-squared-error-of-cross-validation (RMSECV). RMSECV is the square root of the mean of e_m^2 *for samples left out during model estimation*. The calculation of RMSECV can described as follows. In CV a small set of samples are left out during the model identification step and prediction statistics are calculated for the samples left out. Then, the left out samples are returned to the pool and the process is repeated with new samples left out. The process is repeated in a 'round robin' sense until all the samples have been left out once. One of the most common CV strategies is to leave out one sample at a time (e.g., the m^{th} sample) and calculate the model error for the sample left out ($e_{m,\text{cv}} = y_m - x_{m,1}\hat{b}_{1,\text{cv}} + x_{m,2}\hat{b}_{2,\text{cv}} + ... + x_{m,N}\hat{b}_{N,\text{cv}}$) where the $\hat{b}_{n,\text{cv}}$ are estimated from the remaining $M-1$ samples. When many samples are available (e.g., approximately when $M > 30$) the leave-one-out strategy can be a bit optimistic and result in unrealistically low RMSECV. Also, recall that it is of interest to perform CV for the estimation of intraneural damage for new subjects. Therefore leave-one-out CV isn't appropriate for the current analysis because there are multiple (6 to 8) repeat measurements per subject. As a result, the current analysis used a "leave-one-subject-out" CV. This means that approximately 6 to 8 samples are left out in each round of the CV round robin. For example, if there are 27 subjects, the CV approach performs model identification using data from 26 subjects and makes predictions for all measurements for the subject left out and the procedure is continued until each subject has been left out once. For "leave-one-subject-out" CV, RMSECV is an estimate of *prediction* performance for measurements on new subjects. This is in contrast to RMSEC that is a measure of *fit* performance. The distinction is important and cannot be over emphasized; *fit* is easy to do, *prediction* is difficult to do. Leave-one-subject-out CV was used in an effort to best judge model performance based on data from a limited number of subjects.

Significant Results & Findings:

1. There was no correlation between skeletal muscle volume and propensity to healing. This was likely due in part to the role of infection in non-healing and revisions of amputations. Table 2
2. Survey data identified severe pain disturbance affecting the patients' life prior to major amputation. All Veterans enrolled in this study had low physical and mental

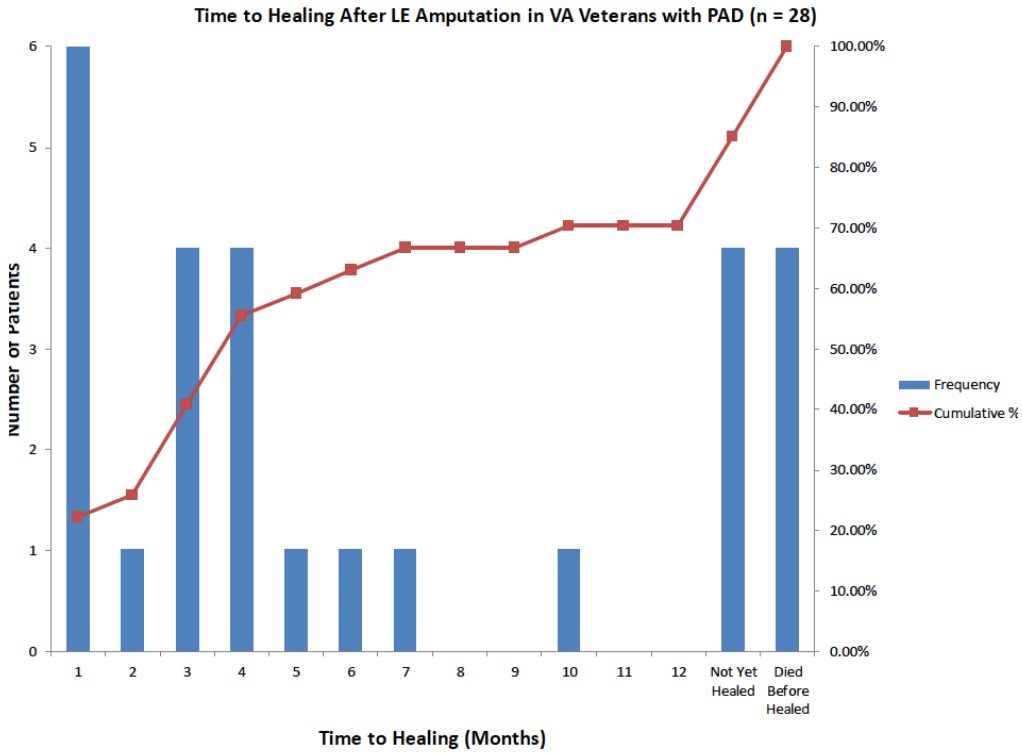
component scores (~30±2 and 42±4) with high pain severity and pain interference scores. Table 2

Table 2 Severe Disability and Pain Scores But No Correlation Between Muscle Area and Healing

	All Patients (n = 16)	Early Wound Healing (n = 7)	Delayed Wound Healing (n = 9)	P-value (Early vs Delayed)
Healing Time, months	**4 ± 0.6**	**2 ± 0.4**	**6 ± 0.6**	**0.0003**
Age, years	66 ± 1	67 ± 2	65 ± 2	0.6
ASA				
III - Severe systemic disease	10/16	4/7	6/9	1
IV - Severe systemic, life-threatening disease	6/16	3/7	3/9	1
Current Smoker	8/16	4/7	4/9	1
HTN	12/16	6/7	6/9	0.6
DM	10/16	4/7	6/9	1
CAD	2/16	1/7	1/9	1
ESRD	5/16	1/7	4/9	0.3
Serum Albumin	2.7 ± 0.1	2.7 ± 0.2	2.6 ± 0.2	0.6
LOS, days	18 ± 3	18 ± 3	18 ± 4	0.9
Hauser Ambulation Index (0-9)	5 ± 0.6	6 ± 0.8	4 ± 0.8	0.1
SF 36 – physical component summary score (0-100)	30 ± 2	27 ± 3	33 ± 3	0.3
SF 36 – mental component summary score (0-100)	42 ± 4	42 ± 6	43 ± 5	0.9
Pain severity (0-10)	7 ± 0.8	7 ± 1	6 ± 1.1	0.6
Pain interference (0-10)	8 ± 0.5	8 ± 0.7	8 ± 0.7	0.4
Amputation revision, same leg	5/16	2/7	3/9	1
Prior amputation, other leg	3/16	1/7	2/9	1
Subsequent amputation, other leg	3/16	2/7	1/9	0.6
Gastrocnemius muscle area, normalized to non-amputated	0.99 ± 0.1 (n=6)	0.94 ± 0.1 (n=3)	1.05 ± 0.1 (n=3)	0.5
Sartorius muscle area, normalized to non-amputated	1.11 ± 0.1 (n=8)	1.01 ± 0.1 (n=4)	1.21 ± 0.1 (n=4)	0.1

3. Veterans segregated into early and late healing groups with a certain proportion of Veterans either not healing or dying before amputation wound was healed (Figure 1).
   There was also a very long LOS after amputation (18 days), a higher than expected revision rate (5/16), and subsequent contralateral amputation rate (3/16) over the 20 months of this study.

Figure 1. Time to Healing after Major Amputation



4. **Patient Demographics.** A total of 30 patients were enrolled at the Atlanta VA. Demographics are presented in Table 3 (Demographics)

Results - Demographics

Age, years, Mean±SE (n)	66.93±1.467 (28)
Follow up, days, Mean±SE (n)	449.5±37.67 (28)
Healing, % (n)	85.7% (24)
Healing Time, days, Mean±SE (n)	96.08±12.20 (24)
Prosthesis Fit, % (n)	75.0% (21)
Time to Prosthesis Fit, days, Mean±SE (n)	148.2±31.73 (21)
Ambulation, % (n)	35.7% (10)
Time to Ambulation, days, Mean±SE (n)	152.5±18.65 (10)
Death, % (n)	21.4% (6)
Time to Death, days, Mean±SE (n)	133.5±29.58 (6)
Post-op Hospitalizations, % (n)	100%, (24)
# of Hospitalization events, , Mean±SE (n)	5.333±0.8569 (24)
Subsequent Amputation, % (n)	17.9% (5)
Time to Subsequent Amputation, Mean±SE (n)	205.2±44.12 (5)

Clinical Data. Table 4 Demographics as they Related to healing (prosthesis fit) and walking with a prosthesis

Results – Association with Primary Outcomes

Univariate regression analysis, Log-rank analysis P-value

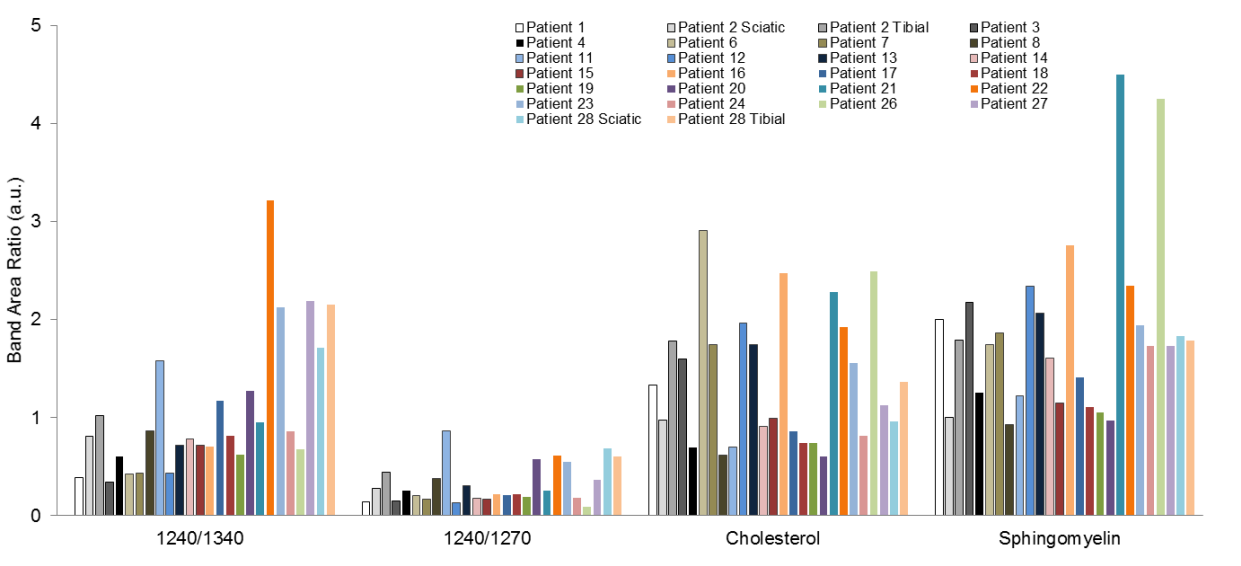
	Time to Healing	Time to Prosthesis Fit	Time to Ambulation
Smoking	0.2117	0.1369	0.0148*
HTN	0.9164	0.9994	0.0213*
ESRD	0.1429	0.0349*	0.9367
Hemodialysis	0.2292	0.0758	0.6910
Renal Transplantation	0.6799	0.4842	0.2265
DM	0.4379	0.1671	0.5750
Insulin	0.1220	0.0254*	0.9782
Active infection	0.2835	0.3355	0.8794
Wet Gangrene	0.1278	0.1529	0.4123
Emergency	0.2242	0.3552	0.3502
Guillotine	0.7080	0.6318	0.2575
Previous amputation	0.7672	0.9287	0.1613
Revision to AKA	0.4998	0.1165	0.7376
Subsequent amp, other limb	0.2386	0.1393	0.9671
NC >255 ABI in amp limb	0.9957	0.7751	0.6155
NC >255 ABI in non-amp limb	0.2997	0.7067	0.1313

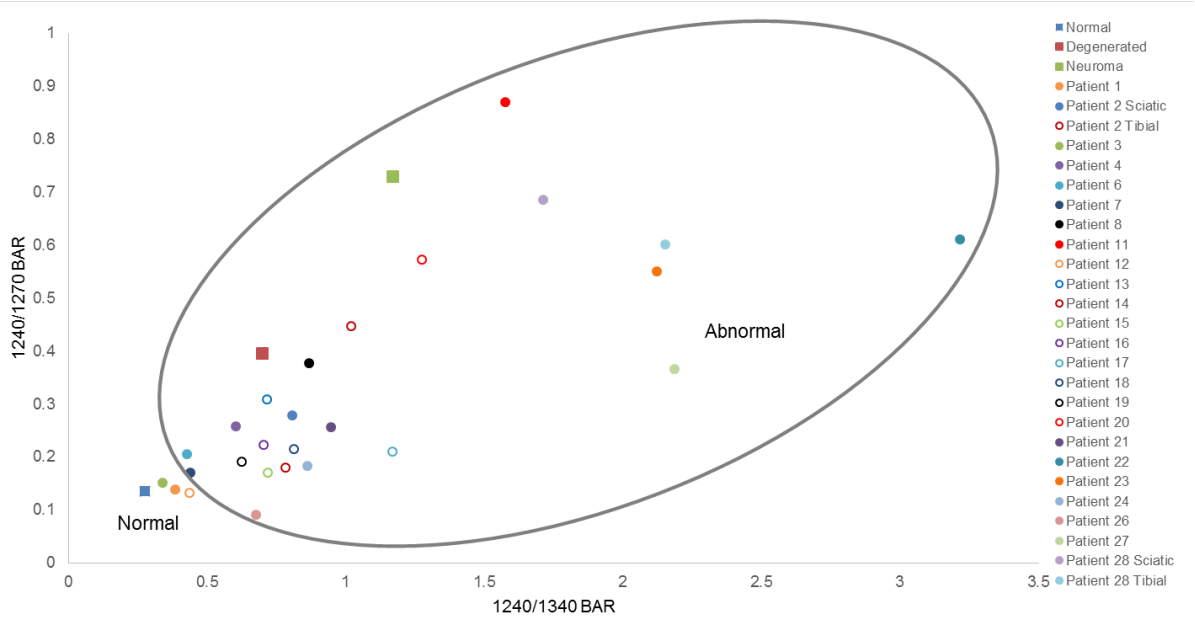
Univariate and Bivariate Exploratory Analysis

In preliminary work, we reported changes in fingerprint region of Raman spectra indicative of demyelination and structural damage in peripheral nerves, damaged by ischemia and traumatic injury. Significant decreases in the Raman spectral band areas of choline and phosphocholine bands [(719+760)/1004 cm^{-1}], free form and cholesterol ester band areas [(608+700)/1004 cm^{-1}], and lipid band areas are evidence of myelin loss. Additionally, we reported changes in Raman spectral bands indicative of the relative disorder of protein within nerve tissue: significantly increased protein-lipid disorder (1240/1270 cm^{-1}), and protein disorder I (1240/1340 cm^{-1}) metrics were associated with neural degeneration. An increase in these BARs may also be described as a decrease in α–helix content relative to total protein and may be markers of axonal death, structural damage, and nerve fibrosis; and are particularly important when examining intact nerve in a clinical setting as outcome is not determined solely by *initial* myelin loss following trauma.

Univariate and bivariate analysis of Raman derived BARs are presented in Figure 2 below for specimens collected from study patients who received amputations. Bivariate comparison of BARs related to protein disorder in Figure 2 are plotted against mean BAR values for normal and abnormal nerves from previously collected data and the ellipsis represents the distribution of previously collected data and served as a guide, but not absolute measure of nerve integrity, to assess the potential success of next-step modeling results. Preliminary grouping, into the normal and abnormal categories based on bivariate plotting of BARs related to protein disorder for Patients 1, 3, 6, 7, 12 and 26 and Patients 2, 11, 20, and 28, respectively, qualitatively correlates with histological fibrosis scores described in Part E and was informative for next-steps. Raman spectral bands used in the calculation of the described BARs were used in initial variable selection of multivariate modeling.

Spectral analysis of cadaver specimens are not pictured in univariate and bivariate results but are included in the subsequent multivariate modeling (Section G) as control specimens.





Histology analysis

Immediately following Raman acquisition nerve samples were fixed in neutral buffered formalin and sent for histological processing including H&E and Masson's Trichrome (MTC) staining[7] as well as Luxol blue/Cresyl violet staining (LFB/CV). Histological scoring, conducted by an outside pathologist, assessed the levels of degeneration, infiltrate, and hemorrhage in each tissue section of nerve. Signs of degeneration included the presence of axonal swelling, dilated myelin sheaths, cellular debris, macrophages, and digestion chambers. Separate scores were assigned for intraneural degeneration, infiltrate type, and intraneural fibrosis using the following system shown in Table 5.

Table 5. Histological Scoring Key developed by outside pathologist

Intraneural Degeneration	**Lymphocytes**	**Macrophages**	**Polyglucosan Bodies**	**Intraneural Fibrosis**
0 - None	0 - None	0 - No macrophages identified	0 - None	0 - None
1 - Evidence of Schwann Cell degeneration in <30% of nerve fibers	1 - Rare, sparse	1 - Rare, sparse macrophages	1 - Rare	1 - Mild fibrosis (<30% staining of intraneural fibers with Masson's Trichrome
2 - Evidence of Schwann Cell degeneration in >30% and <60% of nerve fibers	2 - Moderate or patchy	2 - Frequent macrophages without aggregation	2 - Few identified (1-2/HPF)	2 - >30% and <60%
3 - Evidence of Schwann Cell degeneration in >60% of nerve fibers	3 - Abundant, thickly distributed throughout specimen	3 - Aggregates of macrophages (including granuloma formation)	3 - Frequently identified (>2/HPF)	3 - >60% fibrosis

External pathologist assigned histological scoring of all 26 human nerve samples from patients receiving amputation are presented in Table 6. Representative images of normal and degenerating nerve can be found in Figure 3 for Patients 3 and 20.

For Patient 3, H&E and LFB/CV demonstrates normal nerve features. This is corroborated as in MTC staining, Schwann cells of myelinated fibers stain red, while some pale blue collagenous material is seen between the nerve fibers. Darker blue staining can be seen around the normal epineurial sheath.

Patient 20: H&E staining demonstrates mild degeneration with a sparse lymphocytic infiltrate. Few intact myelinated axons/Schwann cells can be identified. These are recapitulated on LFB/CV and MTC staining. Increased collagen tissue is seen with little residual myelination as indicated by intense blue collagen stains and little red

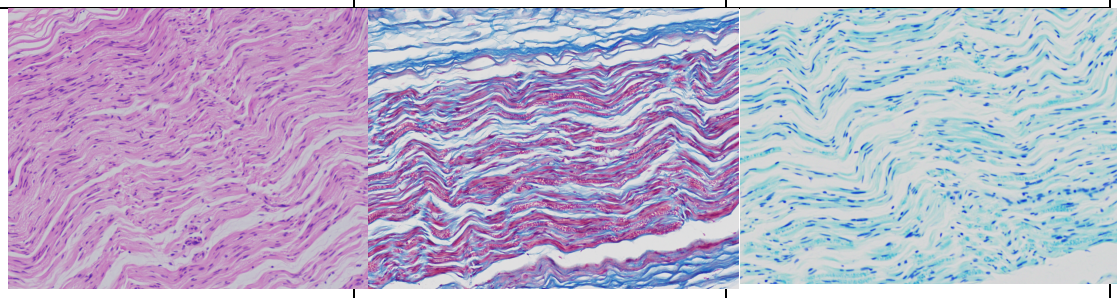
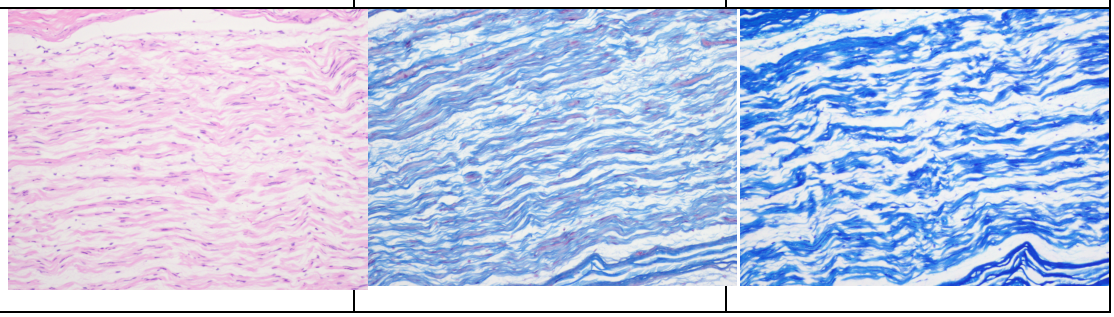
myelination staining in MCT. Both collagen and myelin stain blue for LFB/CV. However, as MTC stain allows us to easily distinguish the collagen scar tissue and residual/regenerating Schwann cells, the blue stained fibers in the LFB/CV images can be attributed to collagen.

Table 6. Histological Scoring of peripheral nerve biopsies from amputated limbs

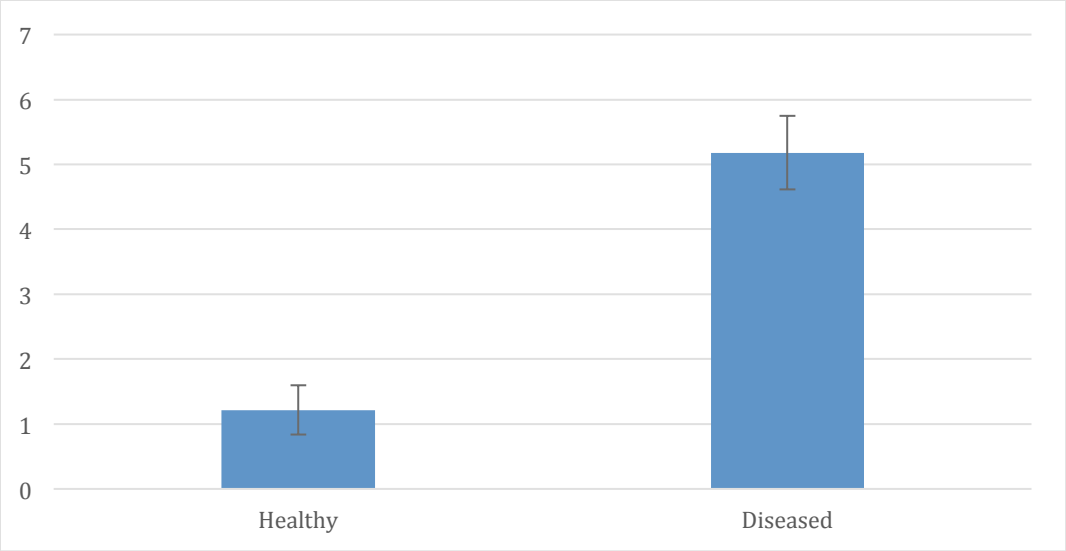
Patient	Nerve	Intraneural Degeneration	Lymphocytes	Macrophages	Polyglucosan Bodies	Intraneural Fibrosis
1	Tibial	1	0	0	0	1
2	Tibial	2	0	0	0	3
2	Sciatic	2	0	0	1	2
3	Tibial	0	0	0	1	0
4	Tibial	2	1	0	0	0
6	Tibial	1	0	0	0	1
7	Tibial	1	1	0	0	1
8	Tibial	3	0	0	0	0
11	Tibial	0	1	0	1	3
12	Tibial	1	2	0	0	1
13	Tibial	0	1	0	0	1
14	Tibial	1	0	1	0	3
15	Tibial	0	0	0	0	3
16	Tibial	0	1	1	0	1
17	Tibial	3	2	1	0	2
18	Tibial	0	0	0	0	0
19	Tibial	2	3	2	0	2
20	Tibial	3	0	1	0	3
21	Tibial	1	0	0	1	1
22	Tibial	1	0	0	0	1
23	Tibial	0	0	0	0	0
24	Tibial	2	1	0	0	1
26	Tibial	0	0	0	0	0

27	Tibial	0	0	0	0	1
28	Tibial	1	0	0	0	3
28	Sciatic	0	0	0	0	2

Figure 3. **Patient 3**: H&E, MTC, and LFB/CV demonstrate normal nerve structures. **Patient 20**: H&E staining demonstrates mild degeneration. MCT staining indicates reduced myelin (red) and increased collagen tissue (blue) and is corroborated by increased blue staining for LFB/CV.

Patient	**H&E**	**Masson's Trichrome**	**Luxol Fast Blue/Cresyl Violet**
3			
20			

Collagen Assay. There was significantly more collagen in the nerves from Veterans who underwent major amputation than the normal controls 5.2 (+/-1.6) mg collagen/gram dry weight of the nerve versus 1.2 (+/-1.2). This exciting finding lends credence to the importance of proper nerve matrix on preventing risk of major amputation. Figure 4.



5. Regression Analysis Relating Raman Measurements to Intraneural Damage *based on histological scoring*. For this study, Raman spectra were available for a total of 35 biopsies corresponding to 24 patients with amputations typically associated with intraneural damage, and 11 cadavers assumed to have no intraneural damage. Six to eight replicate spectra were obtained for each subject and several of the spectra were considered unusual and removed.[2] There were ≥ 4 repeat measurements remaining for each subject. Of the 24 patients with amputations, 18 had intraneural damage scores available and the scores were repeated in the Y-block for the corresponding repeat Raman measurements in the X-block. In this study, intraneural damage was defined as the sum of intraneural degeneration score and intraneural fibrosis score. The intraneural degeneration score and intraneural fibrosis score both ranged from 0 to 3, thus the intraneural damage score ranged from 0 to 6. One patient had an intraneural damage score considered unusual and this data was removed form the analysis.[3] The resulting regression analysis was based on 165 samples ($M = 165$) and 3183 Raman frequencies ($N = 3183$) spanning the range 795 to 1750 cm^{-1}. Cross-validation was performed using a "leave-one-subject-out" data splitting.

Figure 5 shows a plot of the cross-validated prediction of intraneural damage versus the known intraneural damage. [Additional information can be found in the Supplemental Information (SI).] Symbols correspond to different subjects (recall that the cadaver known intraneural damage score was set to zero). The diagonal in Figure 5 is the line of perfect CV prediction (all the points would lie on this line if the CV predictions were perfect and RMSCV was zero). The RMSECV can be interpreted as the standard deviation of the points about the diagonal line. CV prediction is an approximation of future prediction performance and is slightly higher than the fit performance: RMSECV =

[2] The spectra were considered X-block outliers based on Hotelling's T^2 and sum-of-squared residuals {b, c, d. Jackson JE. *A user's guide to principal components*, John Wiley & Sons: New York, NY, 1991}

[3] The score was considered a Y-block outlier based on residuals analysis. {b, c.}

1.15 > RMSEC = 0.98. It is seen in Figure 5 RMSECV is only slightly higher than RMSEC suggests that the PLS model was not over-fit and an approximate F-statistic > 3 (F >3 for both RMSEC and RMSECV). See the SI) both suggest that the observed trend in Figure 5 may be due to a true relationship between Raman measurements and the intraneural damage score. Although this result may be encouraging it is good to keep in mind that the analysis was performed on a small data set (26 total subjects), the error in the intraneural damage score not known, and the conclusion is primarily based on cross-validated prediction instead of on a true independent validation data set. The result is that the hypothesis that Raman measurements can be used to estimate intraneural damage based on histological scores alone is supported but further evidence is required to validate the results.

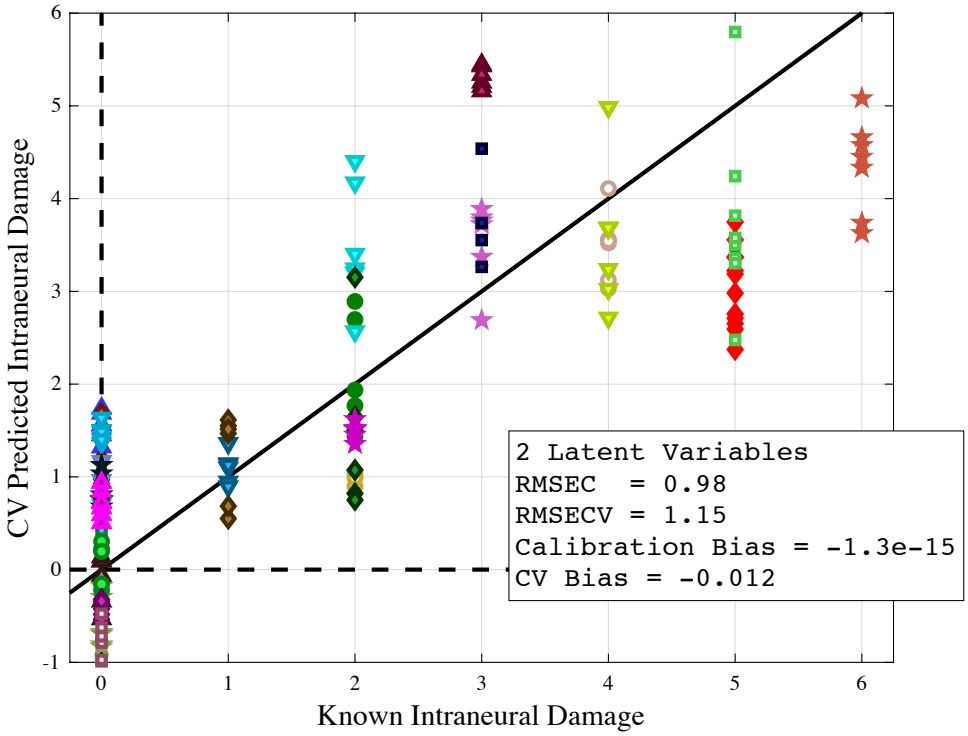


Figure 5. Cross-validation predicted versus measured intraneural damage. Symbols correspond to different subjects. The diagonal corresponds to perfect fit.

Linear multivariate regression analysis was performed to determine if Raman measurements could be used to reliably estimate intraneural damage measured by histological scores alone. The regression model may help understand the fundamental chemistry related to intraneural damage, and provide a fast, objective and inexpensive method for measuring it. Results based on a partial least squares model and leave-one-subject-out cross-validation suggested this may be possible however further evidence and larger sample size is required to reproduce and validate the results.

Major Developments and Conclusions:

Positive findings:

The degree of fibrotic damage to the nerves was not well characterized histologically, but both total collagen content and Raman were able to segregate to these Veterans undergoing major amputation (the majority of whom had diabetes and/or end stage renal disease). Thus, fibrosis correlates with risk of major amputation, and preventing fibrosis in these Veterans may have a profound impact on limb salvage. To date little to no research programs are dedicated to pursuing this critical finding.

Veterans who healed normally (in first 2 months) were more likely to both have prosthetic provided and walk with prosthetic (albeit, most use prosthetic around the house and not in public). Approximately ½ of Veterans do not heal in a normal period of time. This prolonged convalescence likely deconditions the Veterans to a degree that ambulating without focused rehabilitation is unlikely. No Veterans went to acute rehab after their major amputation, so it is likely that a focused rehabilitation that is initiated after the wound is healed could be of much use in promoting ambulation in this population.

Negative findings:

Cross sectional imaging was not useful in predicting wound healing after major amputation.

SF 36 and pain scores were profoundly abnormal in this population but not predictive of ambulation status. This conclusion should be understood within the limitation of rare ambulation rates in this population.

Other achievements:

This work provided vascular services at the Atlanta VAMC (AVAMC) to integrate more closely with our podiatry colleagues, which led to our site participating in a VA CSP study evaluating the role of rifampin antibiotic on wound healing in diabetic patients. This collaboration is dedicated to improving the major amputation rate at our VAMC. This success limited the number of Veterans undergoing major amputation (and time to benchmarks), but is of great utility to our Veterans.

This work provided collaborations, professional development, and building of research infrastructure that was of great impact on persons under Dr. Brewster at the AVAMC and current funding.

Persons:

Lucas Timmins (PhD): Assistant Professor, University of Utah. Department of Biomedical Engineering

Walker Upchurch (BA): now pursuing post graduate education

Hiro Nakahara (MD): now pursuing pathology residency

Andrew Morris (MD): finishing general surgery residency

Julia Raykin (PhD): research scientist, Emory University

Funding:

Dr. Brewster

NIH: RO1. NHLBI.

VA: Intent to fund received for VA RRD SPiRE and BLRD Merit.

Discussion of stated goals not met:

Due to lower than expected number of Veterans undergoing major amputation at AVAMC, we were not able to validate our model relating RAMAN nerve findings prospectively. However, given the strong associations and correlation with collagen content in Veterans undergoing major amputation, we strongly believe that this project provides robust data supporting anti-fibrotic research platforms in peripheral nerves of diabetic Veterans to help prevent major amputation.

We also had a lower than expected wound healing rates. This complicates data analyses and correlations with clinical data. It also provides an opportunity to re-assign rehabilitation to the Veterans after wound healing to target strengthening with the time where the prosthesis will be fit and training provided.

Opportunities for training and professional development project provided:

Mentoring

Provided to the persons listed above.

Conferences

Dr. Nakahara presented data from this project at the annual VA Surgeons meeting in 2018.

Laboratory meetings:

All personnel participated in laboratory meetings with the Brewster laboratory, and Dr. Brewster personally traveled to NRMC at multiple time points to meet with his collaborators on this project.

Results were disseminated to communities of interest:

We have used the data generated from this project to help inform our Veterans of the likely expectations after major amputation as well as to prepare them for the rehabilitation road ahead of them.

Impact:

Impact on development of the principle discipline

The manuscript in preparation will likely be useful in stimulating funding agencies to attack nerve fibrosis as a limb salvage strategy. We are currently organizing data for this publication.

Knowledge/theory research in lay language:

Ambulating after major amputation is difficult in the PAD population. Veterans with diabetes have prolonged healing times, and individualized rehabilitation strategies make logical sense. Preventing amputation in this population may not only lie in providing proper vascular supply but likely in limiting fibrosis of peripheral nerves.

Impact on other disciplines:

This data may be of use in the VA for helping draft rehabilitation guidelines for improving ambulation after major amputation.

Impact on tech transfer

None

Impact on society beyond science and technology

None

Participants and other collaborating organizations

None

Individuals who worked on the project

No Change from quarterly reports

Change in support

Nothing to report

Special Reporting: N/a

Organizations involved as partners

Nothing to Report

Appendices

1. CV (Luke Brewster)

1. Name: Luke P. Brewster, MD, PhD, MA, RVT

2. Office Address: WMB Suite 5105, 101 Woodruff Circle, Atlanta, GA, 30322
   Telephone: 404 727 8329
   Fax: n/a

3. E-mail Address: lbrewst@emory.edu

4. Citizenship: USA

5. Current Titles and Affiliations:

   a. Academic Appointments:

      i. Primary Appointments:
         1. Assistant Professor of Surgery. Emory University, July 2011.
         2. Staff Surgeon, Vascular Surgery. Atlanta VA Medical Center. September 2012.

      ii. Joint and Secondary Appointments:

         1. Institute of Bioengineering and Biosciences
            Georgia Institute of Technology Aug 2011

         2. Staff Scientist, Yerkes National Primate Research
            Center Aug 2011

         3. Scientific Advisory Committee Member
            Atlanta Clinical & Translational Science Institute Sept 2011

         4. Program Faculty, Georgia Institute of Technology
            Bioengineering Program Mar 2012

         5. Program Faculty, Emory's Children's Heart Research and Outcomes Center
            June 2013

         6. Program Faculty, Georgia Institute of Technology and Emory
            Biomedical Engineering Program Dec 2015

   b. Clinical Appointments:

      1. Emory University Hospital Clinical Staff July 2011

2. Atlanta Veterans Affairs Medical Center Clinical Staff July 2011

3. Egleston Children's Hospital Clinical Staff July 2011

c. Other Administrative Appointments:

1. Medical Director, Vascular Laboratory,
Division of Vascular Surgery, The Emory Clinic. Sept 2011

2. Section Chief, Vascular Surgery, Atlanta VAMC Oct 2017

6. Licensures/Boards:

State of Illinois Medical License 2003-current

State of Georgia Medical License 2009-current

7. Specialty Boards:

Registered Vascular Technician 2009

Diplomat American Board of Surgery (ABS) 2010

Diplomat ABS-Vascular Surgery 2012

8. Education:

1992-1997, B.A./B.A. (Biology/Philosophy), Benedictine College; Atchison, KS

1997-2001, M.D. (Distinction in Research), Saint Louis University School of Medicine; Saint Louis, MO

2002-2005, M.A. Bioethics and Health Policy, Loyola University Medical Center (LUMC); Maywood, IL

2003-2006, Ph.D. Cell Biology, Neurobiology, & Anatomy, LUMC; Maywood, IL

9. Postgraduate Training:

2001-2003, LUMC. Intern and second year general surgery resident. Supervisor: Richard L. Gamelli, MD, FACS (Chair)

2002-2005, LUMC, Masters student, Supervisor: Mark Kuczewski, PhD

2003-2006, LUMC, PhD student, Supervisor: Howard P. Greisler, MD, FACS

2006-2009, LUMC, General Surgery resident, Supervisor: Richard L. Gamelli, MD, FACS and Steven DeJong MD, FACS

2009-2011, Emory University School of Medicine, Vascular Surgery Fellowship, Supervisor: Elliot L. Chaikof, MD, PhD, FACS and Thomas F. Dodson, MD, FACS

10. Continuing Professional Development Activities:

Junior Faculty Development Course, Emory University, 2012

11. Committee Memberships:

a. National and International:

American Heart Association, Peripheral Vascular Disease Council

Fellows in Training Program Committee	2012-Present
PAD in Women working group	2012-2016
Member Communications Committee	2015-16
Member, critical limb ischemia writing group	2017-present
Member, Membership committee	2017-present
Chair Early Career committee (PVD council)	2018-present
Member, Vascular Discovery Program Committee	2018-Present
American Board of Surgery	
Ethics content writing group	2012
Association for Academic Surgery	
Publications Committee	2011-2014
Program Committee	2014-6
Association of VA Surgeons	
Research Committee	2015-7
International Society of Applied Cardiovascular Biology	
Abstract Reviewer for 2014 meeting	2013
Awards Committee	2014
Executive Committee	2014-16
Program Committee 2015 meeting	2015
Program Committee 2016 meeting	2014
Chair, Long-term Planning Committee	2016-present
Program Committee 2017 meeting	2017
Program Committee 2018 meeting	2017-present
North American Vascular Biology Organization	

Chair Membership Committee 2018-present

Society for Vascular Surgery

SVS Student and Resident Mentor 2010-Present

Reviewer Medical Student Research Scholarship 2012

SVS Clinical Practice Council 2013-6

SVS Research and Education Committee 2015-present

Chair 2018-2020

SVS Research Council 2017-

SVS Vascular Annual Meeting Program Committee 2018-

Program Committee, annual meeting 2018-

SVS Foundation Board 2018-

Vascular and Endovascular Surgery Society (formerly Peripheral Vascular Surgery Society) Winter Program Committee
2013-15

Chair; Grants & Scholarship Committee 2017-8

Winter Program Committee 2018-present

VA Consultant for Permanent Vascular Repair (DOD) 2017-

b. Institutional:

Emory University Vascular Surgery Fellowship Oversight Committee 2011-Present

Emory University Hospital Ethics' Committee 2011-2017

Emory University School of Medicine MD/PhD Clinical Research Conference 2011-present

Stroke Leadership Committee, Emory University 2012-Present

Hybrid OR Planning Committee 2011-15

Emory Healthcare Clinical Trials Physician Committee 2013

Emory Department of Surgery, Clinical Competency Committee 2013-present

Emory School of Medicine, Director; SubI vascular examination 2014-present

Basic Science Vice-chair, Emory Department of Surgery Research Advisory Council

2014-present

Emory School of Medicine, MD-PhD Advisory Council 2016-present

Surgery representative (young faculty) LCME site visit, Emory SOM 2016

Atlanta VAMC, member Professional Standards Board 2016-present

Atlanta VAMC, member of Prevention of Amputation in Veterans Everywhere Program (PAVE) Committee
2016-present

Emory University, Medical School Research Committee 2018-present

12. Peer Review Activities:

a. Grants:

i. National and International:

Panel #23, NIH Challenge Grants 2009

American Heart Association, Vessel Wall Study Section 2012-6

Italian Ministry of Health, 2013

Bioengineering, Technology, and Surgical Sciences (BTSS) Study Section, NIH 2016

Department of Defense; CDMRP Study Section (ad hoc) 2016, 17

Department of Veteran Affairs, Surgery Study Section (standing member) 2016-8

American Heart Association, Fellowship Study section 2018-

ii. Institutional:

Georgia Tech (GTEC)/Emory Regenerative Medicine Grants
2011,14

Atlanta Clinical and Translational Science Institute (ACTSI) Clinical Interaction Network Scientific Advisory Committee
2011-Present

b. Manuscripts:

Journal of Surgical Research 2012-Present

Annals of Vascular Surgery 2013-Present

Journal American College of Surgeons	2013-Present
Vascular	2015-Present
Journal of Vascular Medicine	2015-Present
Journal of Vascular Surgery	2016-Present
Scientific Reports	2016-Present
Surgery	2016-Present
Acta Biomaterialia	2016-Present
Annals of Biomedical Engineering	2016-7
Arteriosclerosis, Thrombosis, and Vascular Biology	2016
Circulation: Cardiovascular imaging	2016
JAMA-Surgery	2016-Present
Biomechanics and Modeling in Mechanobiology	2017-Present
Journal of American College of Cardiology-Cardiovascular Interventions	2017
Journal of Biomechanics	2017-Present
Journal of Biomedical Materials Research	2017-Present
Journal of the Royal Society of Interface	2017-Present
Circulation Research	2018

c. Conference Abstracts:

i. National and International:

1. International Society of Applied Cardiovascular Biology (ISACB), Biennial Meeting, Cleveland, OH, April 2-5, 2014
2. Association Academic Surgery/Society University Surgeons, Annual Meeting, San Diego, CA, February 4-6, 2014
3. Association Academic Surgery/Society University Surgeons, Annual Meeting, (2014-6)

4. Peripheral Vascular Surgery Society (PVSS), Steamboat Springs, CO, January 30-February 2, 2014
5. Vascular and Endovascular Surgery Society (VESS) [formerly named PVSS], Vail, CO, January 29-Februrary 1, 2015
6. ISACB 2015 meeting, Nuremberg, GE, December 3-4, 2015
7. ISACB 2016 Biennial Meeting, Banff, CA, September 7-10, 2016
8. ISACB 2017 meeting, Cape Town, SA, December 4, 2017
9. American College of Cardiology, Orlando, FL, 2018
10. ISACB 2018 Biennial Meeting, Bordeaux, FR, September 17-20

ii. Regional:

1. Southern Association Vascular Surgery. Scottsdale, AZ. Contralateral Occlusion is not a Clinically Important Reason for Choosing Carotid Artery Stenting for Patients with Significant Carotid Artery Stenosis. January 18-21, 2012.
2. Florida Georgia Vascular Study Group. Atlanta, GA. How Institutional Databases can influence Carotid Therapy and Where They Fall Short. May 9, 2013.
3. Southern Association of Vascular Surgery. Palm Beach, FL. Carotid Artery Stenting has Increased Risk of External Carotid Artery Occlusion Compared to Carotid Endarterectomy. January 15-18, 2014.
4. Midwest Conference on Cell Therapy and Regenerative Medicine. Kansas City, MO. Overcoming limitations with Autologous Cell Therapy in Therapeutic Revascularization. September 18-19, 2015.
5. Vascular Surgery Basic Science Conference. Regenerative Approaches to Critical Limb Ischemia. 2015.
6. Southern Association Vascular Surgery. Cancun, Mexico. A Novel Large Animal Model of Peripheral Arterial Disease. January 20-23, 2016.
7. EPIC-SEC. Atlanta, GA. Value of Limb-salvage over amputation. April 20, 2017.

13. Consultantships/Advisory Boards:
Consultant, Galt Medical 2013

14. Editorships and Editorial Boards:
Editorial Board Member. Journal of Surgical Research. 2014-present

15. Honors and Awards*:*

American Heart Association, Council on PVD

PVD Early Career Investigator Award 2013

American Heart Association, Council on PVD

Jay D. Coffman Early Career Investigator Award 2014

16. Society Memberships:

American College of Surgeons (FACS)

American Heart Association (FAHA)

American Medical Association

Association of Academic Surgery

Georgia Surgical Society

International Society for Vascular Surgery

International Society Applied Cardiovascular Biology

International Surgical Society (Junior Member)

North American Vascular Biology Organization

Society for Vascular Surgery

Society of University Surgeons

Southern Association Vascular Surgeons

Vascular and Endovascular Surgery Society

17. Organization of Conferences:

    a. National and International:

        i. Administrative Positions

- Program Committee, American Heart Association, Peripheral Vascular Disease
- Council's Fellow-in-Training Symposium (at Scientific Sessions), 2012-present
- Winter Program Committee, Vascular and Endovascular Surgery Society 2013-2015.
- Program Committee, Association for Academic Surgery, 2014-2016
- Program Committee, International Society of Applied Cardiovascular Biology, 2015-Present
- Program Committee, Vascular Research Initiatives Conference, 2015-Present
- Conference Co-Organizer (With Dan Simionescu and Tim Pennel), International Society of Applied Cardiovascular Biology, 2017.
- Winter Program Committee, Vascular and Endovascular Surgery Society 2018-
- Program Committee, Society for Vascular Surgery Annual meeting 2018-

        ii. Sessions as Chair:

Co-moderator, Society for Vascular Surgery Annual Meeting. Navigating financial pressures during training and the transition into practice. 2011

Chair, Society for Vascular Surgery Annual Meeting. SVS Trainee Section Program. 2011.

Co-Moderator, Vascular 2: Angiogenesis and Inflammation. 7th Annual Academic Surgical Congress. 2012

Moderator, Ethics Colloquium, American College of Surgeons Clinical Congress. 2012.

Co-Moderator, Session VI. Regenerative Medicine, Hilton Head. 2013.

Co-Moderator, Aortic Aneurysm Session, Fellows in Training (FIT) Symposium, American Heart Association. 2013.

Co-Moderator, Education-open paper session. International Surgery Society. 2013.

Co-Moderator, Scientific Session II, Peripheral Vascular Surgery Society Winter Meeting. 2014.

Co-Moderator, Vascular I, Association for Academic Surgery. 2014.

Co-Moderator, Scientific Session II, Vascular and Endovascular Surgery Society, Winter Meeting. 2015.

Co-Moderator, Session II: Basic/Translation-Endothelial/Cardiovascular Biology, Association of Academic Surgery. 2015.

Moderator, Vascular Surgery 1: Surgical Forum. American College of Surgeons. 2015.

Co-Moderator, Cells and Blood: International Society of Applied Cardiovascular Biology. 2015.

Co-Moderator, Endothelial biology and vascular physiology and clinical. Academic Surgical Congress. 2016.

Co-Moderator, Basic Science: Vascular. Academic Surgical Congress. 2016.

Co-Moderator, Aneurysm. Vascular Research Initiatives Conference. 2016.

Co-moderator, Cardiovascular Devices. European Society Vascular Surgery Spring Meeting. 2016.

Co-moderator, Cardiovascular Remodeling. International Society of Applied Cardiovascular Biology. 2016.

Co-moderator, Aneurysm Management, American College of Surgeons. 2016.

Co-moderator, Ethics Colloquium on Global Surgery, American College of Surgeons. 2017.

Co-moderator, Jay D. Coffman Early Career Investigator Award Competition, American Heart Association. 2017.

Moderator, Cerebrovascular Disease Session of FIT, American Heart Association, PVD Council. 2017.

Co-moderator, Translation session. VRIC. 2017.

Co-moderator, Vascular Biomechanics. ISACB 2018

b. Institutional:

i. Sessions as Chair:

Co-Moderator, Clinical Research. 11th Annual Emory Surgery Research Day. 2012.

Co-Moderator, Basic Science Research, 12th Annual Emory Surgery Research Day. 2013.

18. Clinical Service Contributions:
[Significant Accomplishments]

Emory: Medical director of Vascular Surgery's IAC accredited vascular laboratory; helped establish Emory's carotid and peripheral arterial lab testing criteria; Vascular Surgery lead in hybrid OR updates/new hybrid room. Program lead for current limb salvage center at Emory.

Atlanta VAMC: Represented Surgery in hybrid OR project. Initiated limb salvage collaboration with podiatry service line; vascular surgery section chief.

19. Formal Teaching:

    a. Medical Student Teaching:
       1. Department of Surgery History and Physical Exam Module (Vascular Examination). All surgery sub-I must learn the vascular examination and perform an ankle brachial index. I have led this initiative for our department since 2013. Monthly session. Contact hours vary between 1-4 each session.
       2. Medical student lecture. Peripheral arterial disease, aortic aneurysm, carotid artery disease. 2011-present. Occurrence is once every two months for one hour.
       3. Department of Surgery Surgical bootcamp. Anastomosis lecture and practicum. 2011-present. 4 hours annually.
    b. Graduate Programs:
       i. Residency Programs:
    a. Vascular surgery topics. 2011-Present. 1 hour sessions. ~1 per year.
       ii. Fellowship Programs:
    a. Vascular ultrasound series. 2011-Present. 1 hour. Monthly.
    b. Vascular Basic and Translational Sciences lecture. 1 hour bi-monthly.
       iii. Master's and PhD Programs:
    a. Vascular needs for Robotics students (Georgia Tech). 1 hour. Twice a year.

20. Supervisory Teaching:
    a. PhD Students Directly Supervised: *[Name, years, current position]*
       1. Alex Cetnar. 2017-current. 1st year PhD student.
    b. Postdoctoral Fellows Directly Supervised:
       [Name, years, current position]
       1. Anastassia Pokutta. 2015-6. Instructor Georgia Institute of Technology.
       2. Lucas Timmins. 2015-6. Assistant Professor University of Utah

    c. Residency Program:
       [Name, years, current position]
       1. Tatiana Chadid, 2014-15. Chief Resident General Surgery, Emory. Will start vascular surgery fellowship this fall.
       2. Andrew Morris, 2015-17. General Surgery Resident Emory. PGY 3.
    d. Thesis Committees: *[Name, program, institution, year]*

       1. Ruoya Wang (GTEC): Dissertation Committee
       2. Parisa Pooyan (GTEC): Dissertation Committee
       3. Daniel Tanner (GTEC): Masters' Thesis Committee
       4. Julia Rankin (GTEC): Dissertation Committee
       5. Rachel Simmons (GTEC/Emory): comprehensive committee
       6. Mario Martinez (GTEC/Emory): Dissertation Committee

7. Torri Rinker (GTEC): Dissertation Committee
8. Katy Lassahn (GTEC): Masters' Thesis Committee
9. Claire Segar (GTEC): Dissertation Committee
10. Mahdi Hasani (GTEC): Dissertation Committee

e. Other:
[Name, program, institution, year]

1. Robert Beaulieu Discovery Module, Emory SOM. 2011

   currently general surgery resident at Johns Hopkins

2. Dina Shaher Itum Discovery Module, Emory SOM. 2012

   Currently general surgery resident UT-Southwestern; AHA Stroke Research Award

3. Scott Robinson Discovery Module, Emory SOM. 2014

   currently vascular resident at Michigan; American Medical Association Medical Student Research Award; Society for Vascular Surgery (SVS) Medical Student Research Award; American College of Surgeons, Medical Student Poster Award 3rd place; Emory Department of Surgery, Basic Science Poster Award 1st place

4. Josh Preiss: Discovery Module, Emory SOM. 2015

   General surgery resident at University of North Carolina

7. Siddarth Dalal: M2 Summer Student, Mercer College of Medicine. 2015.
8. Nicholai Henry: M4 Morehouse SOM. SVS Medical Student award. 2017.

21. Lectureships, Seminar Invitations, and Visiting Professorships:

a. National and International:

45th Walter Reed Vascular Surgery Symposium. Bethesda, MD. Novel solutions and translational research platforms to meet the increasing needs of PAD patients. Harris B Shumacker Lecture. September 18, 2017.

b. Regional:

Regenerative Approaches to PAD patients. Greenville Health System/Clemson University. Harriet and Jerry Dempsey Research Conference. Greenville, SC. September 30, 2016.

c. Institutional:

1. Current limitations to healing after vascular interventions and potential inroads. Emory SOM; Department of Surgery Grand Rounds. July 14, 2011.
2. Carotid artery stenosis: current screening and treatment recommendations. Emory

SOM; Heart and Vascular Grand Rounds. January 27, 2014.
3. Carotid artery stenosis contemporary trials, mechanistic insights, and the Emory experience. Emory SOM; Department of Neurology Grand Rounds, October 7, 2016.
4. Novel Animal Models of PAD. Cardiovascular Research Seminar (Emory Cardiology). February 16, 2018.

22. Invitations to National/International, Regional, and Institutional Conferences:

a. National and International:

1. Paracrine and Stromal Effects of Ischemic Human MSCs on Endothelial Cells in 3D culture. TERMIS (Tissue Engineering International & Regenerative Medicine Society)-Americas. Atlanta, GA. November 10-13, 2013.
2. Ethics and Disclosure for the Young Surgeon. American College of Surgeons. Chicago, IL. October 4-8, 2015.
3. Interventional Outcomes in PAD. American Heart Association, Scientific Sessions. Orlando, FL. November 7-11, 2015.
4. Robust Angiogenic Potential of Mesenchymal Stem Cells is Preserved in Patients with Critical Limb Ischemia. International Society of Cardiovascular Biology. Nuremberg, Germany. Session Keynote Lecture. December 3-4, 2015.
5. Panelist-Professional Development and Mentorship in Academic Practice. PVD Council Lunch Meeting. American Heart Association, Arteriosclerosis Thrombosis Vascular Biology/Peripheral Vascular Disease (PVD) Meeting. Nashville, TN. May 5-7, 2016.
6. Changing landscape of lower extremity intervention. American Heart Association, Scientific Sessions. New Orleans, LA. November 12-16, 2016.
7. Preparing for job negotiations. American Heart Association, Scientific Sessions. PVD Council FIT Symposium. Anaheim, CA. November 11-15, 2017.
8. Translational Models of PAD—a voyage or an important destination. International Society of Applied Cardiovascular Biology. Cape Town, South Africa. December 2-4, 2017.

23. Abstract Presentations at National/International, Regional, and Institutional Conferences:

a. National and International:

1. Femoral Artery Stiffness Correlates with Circumferential Collagen Alignment. (Poster) Regenerative Medicine, Harnessing Biology for Regeneration. Hilton Head, SC. 2012.
2. Quantifying differences in local wall stress between carotid and femoral arteries. (Poster) Experimental Biology, San Diego, CA. 2012.
3. *Carotid Artery Stenting Does Not Increase Risk of External Carotid Artery Occlusion Compared to Carotid Endartectomy. (Oral) International Surgical Week. Helsinki, Finland. August 25-29, 2013.
4. The Influence of the Hostile Neck on Restenosis after Carotid Stenting. (Oral) Peripheral Vascular Surgery Society. Steamboat Springs, CO. January 29-February 2, 2014.
5. Defining ultrasound criteria for significant ipsilateral carotid stenosis in patients with contralateral carotid artery occlusion. (Oral) Association for Academic Surgery. San Diego, CA. February 4-6, 2014.

6. *Successful rejuvenation of mesenchymal stem cells from amputated limbs of vascular patients for therapeutic applications. (Oral) American Heart Association, ATVB meeting. Toronto, Canada. April 30-May 3, 2014.
7. *Disturbed flow stiffens murine carotid arteries through proinflammatory collagen deposition and remodeling. (Oral) American Heart Association, Scientific Sessions. Chicago, IL. Jay D. Coffman Award Presentation. November 15-19, 2014.
8. Late mortality in females after endovascular aneurysm repair: effect of preoperative aneurysm size. (Oral) Association of Academic Surgery. Houston, TX. February 5-8, 2015.
9. Individualized differences in mesenchymal stem cells' invasion does not impact in vitro endothelial cell angiogenic activity in diabetic patients with critical limb ischemia. (Oral) Association of VA Surgeons. Miami, FL. May 3-5, 2015.
10. High Glucose does not inhibit aortic endothelial cell sprouting and stimulates proliferation. (Oral) Society of University Surgeons. Jacksonville, FL. February 2-4, 2016.
11. *Characterization of Hemodynamic Environment and Arteriogenesis in a Large Animal Model of Peripheral Arterial Disease. (Oral) Regenerative Medicine. Hilton Head, SC. March 16-19, 2016.
12. *Molecular Mechanism of Disturbed Flow in Arterial Stiffening. (Oral) Vascular Research Initiative Conference. Nashville, TN. May 4, 2016.
13. *Impact of Aging on Murine Elastic and Muscular Arteries. (Oral) European Society Vascular Surgery. London, England. May 13-14, 2016.
14. *Diabetic Mesenchymal Stem Cells Promote Endothelial Cell Invasion through Unique Angiogen Pathways. (Oral) Society of University Surgeons. Las Vegas, NV. February 7-9, 2017.

24. Research Focus:

My laboratory investigates the biomechanical mechanisms that contribute to pathologic vessel remodeling in peripheral vascular disease, develops regenerative strategies for use in ischemic tissue, and works to improve the function of patients who succumb to major amputation.

25. Patents:

    a. Issued: Luke Brewster, Scott Robinson, Ian Copland. 15/449,067; Emory 15101 US. Compositions Derived from Platelet Lysates and Uses in Vascularization.

26. Grant Support:

    a. Active Support:
        i. Federally Funded:

            a. PI, National Institutes of Health, NHLBI, Molecular mechanisms of flow-dependent arterial remodeling in PAD. 8/2018-7/2023

b. PI, National Institutes of Health, NHLBI, Molecular Mechanism of Disturbed Flow in Arterial Stiffening, KO8HL119592, $160,812/year; 4/14-1/19
c. PI, Department of Defense, CDMRP/OPORP; Raman Spectroscopy and 3D Imaging as Decision Support Tools in the Assessment of Neuronal Fibrosis and Sarcopenia in Veterans and Combat Casualty Amputees. OP140015, $434,910 (Total Award). 9/15-8/17 NCE (2018)
d. Co-I, NHLBI R61HL138657 (PI Quyyumi)

e. Private Foundation Funded:

1. PI. Matching award for KO8. SVS Foundation/American College of Surgeons Mentored Clinical Scientist Research Career Development Award. $250,000 (Total Award) 4/14-1/19.

2. Co-I. American Heart Association, X-ray phase contrast CT based analysis of atherosclerotic plaques. 16GRNT30860004, $77,000/year. PI (Xiangyang Tang, Radiology, Emory) 7/16-6/19.

b. Previous Support:

1. Preconditioning Human MSCs from Amputated Ischemic Limbs for Autologous Clinical Application. Emory Georgia Institute Technology Regenerative Engineering and Medicine (REM) Seed Grant. $50,000. 2012.
2. SAPPHIRE WW: Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy: Source: Cordis; Emory Site PI. 2011-14.
3. Evaluation of the Gore Conformable TAG Thoracic Endoprothesis for the Treatment of Acute Complicated Type B Dissections. Source: W. L. Gore & Associates, Inc. Emory Site PI.2011-13.
4. Angiogenic Potential of Adipose Tissue-derived Mesenchymal Stem Cells from Ischemic Limbs. American Heart Association, IRG14740001 ($150,000) 1/13-12/14
5. Pre-Investigative New Drug Delivery Testing of Autologous MSCs in Plasma Gel Vehicle in a Porcine Model of Critical Limb Ischemia. Emory Georgia Institute Technology Regenerative Engineering and Medicine (REM) Seed Grant ($70,000) 9/13-8/14 (NCE-3/15)
6. The Impact of Weight Loss on Arterial Health in Morbidly Obese Adolescents. Emory Children's Center for Cardiovascular Biology ($50,000) 9/13-7/15
7. Unite-A Phase II, Single-Arm Prospective Study of the Safety and Efficacy of the UniFit Aorto-uni-iliac (AUI) Endoluminal Stent Graft for the Repair of Abdomial Aortic Aneurysms in Patients who are not Candidates for Repair with Commercially Available Bifurcated Endovascular Prostheses or Conventional Surgical Repair. Duke Emory Site PI 2013-5
8. ANGES A phase 3 double-blind, randomized, placebo-controlled study to evaluate

the safety and efficacy of AMG0001 in subjects with critical limb ischemia. Emory Site PI 2015-2016

27. Bibliography:

a. Published and Accepted Research Articles (clinical, basic science, other) in Refereed Journals:

1. **Brewster LP,** Brey EM, Tassiopoulos AK, Xue L, Greisler HP. The FGF-1 Based S130K-HBGAM Mutant Chimera is a Heparin Independent EC and SMC Mitogen. American Journal of Surgery, 2004;188:575-579.

2. Labropoulos N, Leon L, **Brewster LP**, Pryor L, Tiongson J, Kang SS, Mansour MA, Kalman P. Are Your Arteries Older Than Your Age? European Journal of Vascular and Endovascular Surgery, 2005;30:588-596.

3. **Brewster LP**, Risucci DA, Joehl RJ, Littooy FN, Temeck BK, Blair PG, Sachdeva AK. Management of Adverse Surgical Events: A structured education module for residents. American Journal of Surgery. 2005;190:687-690.

4. **Brewster LP,** Bennett B, Gamelli RL. Application of Rehabilitation Ethics to a Selected Burn Patient Population's Perspective. J Am Coll Surg. 2006; 203:766-771.

5. **Brewster LP**, Brey EM, Addis M, Xue L, Husak V, Ellinger J, Greisler HP. Improving Endothelial Healing with Novel Chimeric Mitogens. Am J Surg. 2006;192:589-593.

6. **Brewster LP**, Washington C, Brey EM, Maddox E, WH Velander, Burgess WH, Greisler HP. Construction and Characterization of a FGF-1 Mutant-Collagen Binding Domain Chimera That Binds Collagen and Stimulates Endothelial Cell Proliferation and Chemotaxis. Biomaterials. 2008;29:327-336.

7. **Brewster LP,** Risucci DA, Joehl RJ, Littooy FN, Temeck BK, Blair PG, Sachdeva AK. Comparison of resident self-assessments with trained faculty and standardized patient assessments of clinical and technical skills in a structure educational module. Am J Surg. 2008; 95:1-4.

8. **Brewster LP,** Trueger N, Schermer C, Ghanayem A, Santaniello J. Infraumbilical Anterior Retroperitoneal Exposure of the Lumbar Spine in 128 Consecutive Patients. World J Surg. 2008;32:1414-1419.

9. Gresik C, **Brewster LP**, Abood G, Supple K, Silver G, Nickoloff B, Gamelli RL. Ecthyma Gangrenosum following Toxic Epidermal Necrolysis Syndrome in a 3 year old boy—a survivable series of events. J Burn Care Res. 2008;29(3):555-558.

10. Moalem J, Salzmann P, Ruan DT, Cherr GS, Freiburg C, Farkas RR, **Brewster LP**, James TA. Should all duty hours be the same? Results of a national survey of surgical trainees. Journal of the American College of Surgeons. 2009;209(1):47-54.

11. **Brewster LP,** Ucuzian AA, Brey EM, Liwanag M, Samarel AM, Greisler HP. FRNK overexpression limits the depth and frequency of vascular smooth muscle cell invasion in a three-dimensional fibrin matrix. Journal of Cellular Physiology. 2010;225(2):562-568.

12. Ucuzian AA, **Brewster LP,** East AT, Pang Y, Gassman AA, Greisler HP. Characterization of the chemotactic and mitogenic response of SMCs to PDGF BB and FGF2 in fibrin hydrogels. Journal of Biomaterials Research Part A. 2010;94(3):988-996.

13. Thirunavukarasu P, **Brewster LP,** Pecora SM, Hall DE. Educational intervention is effective in improving knowledge and confidence in surgical ethics—a prospective study. Am J. Surgery,2010;200:665-669.

14. **Brewster LP,** Beaulieu R, Salam A, Veeraswamy R, Dodson TF, Kasirajan K. Carotid revascularization outcomes comparing distal filters, flow reversal, and endarterectomy. Journal of Vascular Surgery, 2011;54(4)1000-1005.

15. **Brewster LP,** Hall DE, Joehl RJ. Assessing Residents in Surgical Ethics: we do it a lot; we only know a little. Journal of Surgical Research, 2011;171(2):395-398.

16. **Brewster LP,** Palmatier J, Manley CJ, Hall DE, Brems JJ. Liver recipients place limits on the power of surrogate decision-makers. Journal of Surgical Research, 2011;172(1):48-52.

17. De Martino RR, **Brewster LP,** Kokkosis AA, Glass C, Boros M, Kreishman P, Kauvar D, Farber A. The perspective of the vascular surgery trainee on new ACGME regulations, fatigue, resident training, and patient safety. Vascular and Endovascular Surgery, 2011;45:697-702.

18. **Brewster LP,** Beaulieu R, Patel S, Kasirajan K, Corriere MA, Ricotta JJ II, Dodson TF. Contralateral Occlusion is not a Clinically Important Reason for Choosing Carotid Artery Stenting for Patients with Significant Carotid Artery Stenosis. Journal of Vascular Surgery, 2012;56:1291-4.

19. Defreitas DJ, Love TP, Kasirajan K, Haskins NC, Mixon RT, **Brewster LP,** Duwaryi Y, Corriere MA. Computed tomography angiography-based evaluation of great saphenous vein conduit for lower extremity byass. Journal of Vascular Surgery, 2013;57(1): 50-55.

20. Martin BM, Love TP, Srinivasan J, Sharma J, Pettitt BJ, Sullivan C, Pattaras J, Master VA, **Brewster LP**. Designing an Ethics Curriculum to Support Global Health Experiences in Surgery. Journal of Surgical Research, 2014;187(2):367-70.

21. Wang R, **Brewster LP**, Gleason RL. In-situ characterization of the uncramping process of arterial collagen fibers using two-photon confocal microscopy and digital image correlation. Journal of Biomechanics, 2013; 46(15):2726-9.

22. Wang R, Raykin J, Li H, gleason RL, **Brewster LP**. Differential Mechanical Response and Microstructural Organization between Non-human Primate Femoral

and Carotid Arteries. Biomechanics and Modeling in Mechanobiology; 2014; 13(5):1041-51.

23. Ruddy JM, Reisenman P, Priestley J, **Brewster LP**, Duwaryi Y, Veeraswamy R. Stent-graft Therapy for False lumen Aneurysmal degeneration in established type B aortic dissection Results in Differential volumetric remodeling of the thoracic versus abdominal aortic segments. Annals of Vascular Surgery, 2014; 28(7):1602-9.

24. Rajani RR, Johnson LS, Brewer BL, **Brewster LP**, Duwaryi Y, Reeves JG, Veeraswamy RK, Dodson TF. Anatomic characteristics of aortic transection: centerline analysis to facilitate graft selection. Annals of vascular surgery, 2014;28(2):433-6.

25. Brown KA, Itum DS, Reeves JG, Duwaryi Y, Rajani R, Veeraswamy RK, Arya S, Salam A, Dodson TF, **Brewster LP**. Influence of the hostile neck on restenosis after carotid stenting. Annals of Vascular Surgery, 2015;29(1):9-14.

26. Brown KA, Itum DS, Reeves JG, Duwaryi Y, Rajani R, Veeraswamy RK, Arya S, Salam A, Dodson TF, **Brewster LP**. Carotid artery stenting has increased risk of external carotid artery occlusion compared to carotid endartectomy. Journal of Vascular Surgery, 2015;61(1):119-24.

27. JE Preiss, Itum DS, Reeves JG, Duwaryi Y, Rajani RR, Veeraswamy RK, Salam A, Dodson TF, **Brewster LP**. Carotid duplex criteria for patients with contralateral occlusion. Journal of Surgery Research, 2015;193(1):28-32.

28. Huen KH, Chowdhury R, Shafii SM, **Brewster LP,** Arya S, Duwaryi Y, Veeraswamy RK, Dodson TF, Rajani RR. Smoking Cessation is the least successful outcome of risk factor modification in uninsured patients with symptomatic peripheral arterial disease. Annals of Vascular Surgery, 2015;29(1):42-49.

29. Arya S, Kim SI, Duwaryi Y, **Brewster LP,** Veeraswamy R, Salam A, Dodson TF. Frailty increases the risk of 30-day mortality, morbidity, and failure to rescue after elective abdominal aortic aneurysm repair independent of age and comorbidities. Journal of Vascular Surgery, 2015;61(2):324-31.

30. L Cendales, R Bray, H Gebel, **L Brewster**, R Elbein, D Farthing, M Song, D Parker, A Stillman, T Pearson, AD Kirk. Tacrolimus to Belatacept Conversion Following Hand Transplantation: A Case Report. American Journal of Transplantation, 2015;15(8);2250-5.

31. D Polhemus, J Bradley, K Islam, **LP Brewster,** J Calvert, Y Tao, C Chang, I Pipinos, T Goodchild, D Lefer. Therapeutic potential of sustained release sodium nitrite for critical limb ischemia in the setting of metabolic syndrome. Am J Physiol Heart Circ Physiol, 2015;309(1);H82-92.

32. K Islam, D Polhemus, Erminia Donnarumma, **L Brewster**, D Lefer. Hydrogen sulfide levels and nuclear factor-erythroid 2-related factor 2 (NRF2) activity are attenuated in the setting of critical limb ischemia. In Press; Journal of the American Heart Association, 2015, 4(5). Doi: 10.1161/JAHA.115.001986.

33. JM Bradley, KN Islam, DJ Polhemus, E Donnarumma, **LP Brewster**, Y Tao, TT Goodchild, DJ Lefer. Sustained release nitrite therapy results in myocardial protection in a porcine model of metabolic syndrome with peripheral vascular disease. Am J Physiol Heart Circ Physiol, 2015;309(2):H305-17.

34. JE Preiss, S Arya, Y Duwaryi, SM Shafii, RK Veeraswamy, RR Rajani, TF Dodson, **LP Brewster**. Late mortality in females after endovascular aneurysm repair. J Surg Res, 2015;198(2);508-14.

35. Brahmbhatt R, **Brewster LP**, Shafii S, Rajani RR, Veeraswamy R, Salam A, Dodson TF, Arya S. Gender and frailty predict poor outcomes in infrainguinal vascular surgery. 2016; 201(1):156-65.

36. Hoffman JR, Chowdhury R, Johnson LS, **Brewster LP**, Duwaryi Y, Reeves JG, Veeraswamy RK, Dodson TF, Rajani RR. Posttraumatic resuscitation affects stent graft sizing in patients with blunt thoracic aortic injury. Am Surg, 2016;82(1):75-78.

37. Robinson ST, Douglas AM, Chadid T, Kuo K, Rajabalan A, Li H, Copland IB, Barker TH, Galipeau J, **Brewster LP**. A novel platelet lysate hydrogel for endothelial cell and mesenchymal stem cell-directed neovascularization. Acta Biomater, 2016;36:86-98.

38. Flink BJ, Long CA, Duwaryi Y, **Brewster LP**, Veeraswamy R, Gallagher K, Arya S. Women undergoing aortic surgery at higher risk for unplanned readmissions compared with men especially when discharged home. Journal of Vascular Surgery 2016;63(6):1496-1504.

39. Arya S, Long CA, Brahmbhatt R, Shafii S, **Brewster LP**, Veeraswamy R, Johnson TM, Johanning JM. Preoperative frailty increases risk of non-home discharge after elective vascular surgery in home-dwelling patients. Ann Vasc Surg, 2016; 35:19-29.

40. Duwaryi Y, Goss J, Knechtle W, Veeraswamy RK, Arya S, Rajani RR, **Brewster LP**, Dodson TF, Sweeney JF. The readmission event after vascular surgery: causes and costs. Ann Vasc Surg, 2016; 36:7-12.

41. **LP Brewster,** ST Robinson, R Wang, S Griffiths, H Li, A Peister, IB Copland, TM McDevitt. Expansion and Angiogenic Potential of Mesenchymal Stem Cells from Patients with Critical Limb Ischemia. Journal of Vascular Surgery, 2017;65(3):826-838.

42. Long CA, Timmins LH, Koutakis P, Goodchild TT, Lefer DJ, Pipinos II, Casale GP, **Brewster LP**. An endovascular model of ischemic myopathy from peripheral artery disease. Journal of Vascular Surgery, 2017:66(3):891-901.

43. Wang R, Raykin J, **Brewster LP**, Gleason RL Jr. A novel approach to assess the in situ versus ex vivo mechanical behaviors of the coronary artery. J Biomech Eng. 2017; 139(1).

44. Prologo JD, Gilliland CA, Miller M, Harkey P, Knight J, Kies D, Hawkins CM, Corn D, Monson DK, Edalat F, Dariushnia S, Brewster L. Percutaneous

image-guided cryoablation for the treatment of phantom limb pain in amputees: a pilot study. JVIR. 2017:28(1):24-34.

45. Morris AD, Preiss JE, Ogbuchi S, Arya S, Duwaryi Y, Dodson TF, Jordan WD, **Brewster LP**. Longer patient travel times associated with decreased follow-up after EVAR. American Surgeon. 2017;83(8):339-341.

46. Kim, CW, Pokutta-Paskaleva A, Kumar S, Timmins LH, Morris AD, Kang DW, Dalal S, Chadid T, Kuo KM, Raykin J, Li H, Yanagisawa H, Gleason RL Jr., Jo H, **Brewster LP**. Disturbed flow promotes arterial stiffening through thrombospondin-1. Circulation. 2017;136(13):1217-32.

47. Salvo NL, Walsh MD, **Brewster LP**. Promoting limb salvage through multi-disciplinary care of the diabetic patient. Current Treatment Options Cardiovascular Medicine. 2017;19(7):55.

48. Arya S, Binney ZO, Khakharia A, Long CA, **Brewster LP**, Wilson PW, Jordan WD Jr., Duwaryi Y. High hemoglobin A1C associated with increased adverse limb events in PAD patients undergoing revascularization. Journal of Vascular Surgery. 2018;67(1):217-228.

49. Arya S, Khakharia A, Binney ZO, DeMartino RR, **Brewster LP**, Goodney PP, Wilson PWF. Statins have a dose-dependent effect on amputation and survival in PAD patients. Circulation. 2018. Epub ahead of print. Doi: 10.1161/CIRCULATIONAHA.117.032361.

50. Arya S, Binney Z, Khakharia A, **Brewster LP**, Goodney P, Patzer R, Hockenberry J, Wilson PWF. Race and socioeconomic status independently affect risk of major amputation in PAD. JAHA. 2018;7(2). Doi: 10.1161/JAHA.117.007425.

51. Morris AD, Dalal S, Li H, **Brewster LP**. Human diabetic mesenchymal stem cells from peripheral arterial disease patients promote angiogenesis through unique secretome signatures. Surgery. 2018;163(4):870-76.

52. Chadid T, Morris AD, Surowiec A, Robinson S, Sasaki M, Galipeau J, Pollack BP, **Brewster LP**. Reversible defects in diabetic MSCs from peripheral arterial disease patients. In Press. Journal of Vascular Surgery.

b. Review Articles:

1. **Brewster LP**. 2004 Some Ethical Issues with Placebo Operations. Virtual Mentor. Available at: http://www.ama-assn.org/ama/pub/category/13081.html Accessed October 1.

2. **Brewster LP**, 2005. Gamelli RL. Improving Clinical Outcomes in Trauma-related Hemorrhage: cleaning up a bloody mess. Issues in Hemostasis Management.1(3):1-10.

3. **Brewster LP,** Brey EM, Greisler HP. 2006.Cardiovascular gene delivery: the good road is awaiting. Advanced Drug Delivery Reviews.58:604-629.

4. **Brewster LP,** Bufallino D, Ucuzian A, Greisler HP. 2007. Growing a living blood vessel: insights for the second hundred years. Biomaterials. 28:5028-5032.

5. **Brewster LP,** Kasirajan K. 2011. Thoracic endovascular aneurysm repair for thoracic aneurysms; what we know what to expect. Annals of Vascular Surgery; 25(6):856-65.

6. Kumar V, **Brewster LP,** Caves J, Chaikof E. 2011. Tissue engineering of blood vessels: current status, requirements, and future challenges. Cardiovascular Engineering and Technology, 2(3):137-48.

7. Johnson CJ, **Brewster LP.** 2013. Carotid Artery Intima Media Thickness (IMT) and the Renin Angiotensin System (RAS). Hospital Practice; 41(2):54-61.

8. Prologo JD, Hawkins M, Gilliland C, Chinnadurai R, Harkey P, Chadid T, Lee Z, **Brewster L**. 2016. Interventional stem cell therapy. Clin Radiol. 2016;71(4): 307-11.

c. Book Chapters:

1. Leon L, **Brewster LP**, Labropoulos N. Noninvasive screening and utility of carotid intima-media thickness. In: Labropoulos N., Mansour MA, eds. *Vascular Diagnosis*. Philadelphia: Elsevier Publishing Co, 2004: 157-74.
2. **Brewster LP,** Brey EM, Greisler HP. Blood vessels. In: Lanza R, Langer R, &Vacanti J, eds. *Principles of Tissue Engineering.* New York: Elsevier Publishing Co, 2006: 569-584.
3. **Brewster LP,** Greisler HP. Advances in tissue engineered vascular grafts. Online Update to Vascular Grafts; Chapter 39. IN: Rutherford RB *Vascular Surgery*. New York: Elsevier Publishing Co, 2006. http://www.vascularsurgerytext.com/content/default.cfm.
4. **Brewster LP,** Chaikof EL. Acute and Chronic Mesenteric Ischemia. IN: *Cohn SM & S Brower eds. EBM in Elective General Surgery.* Peoples Medical Publishing House, Inc. USA. 2011.
5. **Brewster LP,** Corriere MC. Surgical Therapy for Femoral-Popliteal Disease. IN: *Dodson TF, Lubin M, Winawer N. eds. Medical Management of the Surgical Patient 5th edition.* Cambridge University Press, Cambridge, UK.
6. **Brewster LP,** Kasirajan K. Chronic Mesenteric Ischemia. IN: *Dodson TF, Lubin M, Winawer N. eds. Medical Management of the Surgical Patient 5th edition.* Cambridge University Press, Cambridge, UK.
7. **Brewster LP,** Greisler HP. Vascular Grafts. IN: Moore WS, eds. Vascular and Endovascular Surgery, 8th edition. Elsevier Press.
8. **Brewster LP,** Brey EM, Greisler HP. Blood Vessels. IN: Lanza eds. Principles of Tissue Engineering, 4th edition, Academic Press. Chapter 39;pp. 795-811.

9. Ruddy JM, **Brewster LP.** Femoropopliteal bypass with reversed saphenous vein. IN: Mansour, Bismuth, Mitchell, Shames; eds. Atlas of Vascular and Endovascular Surgical Techniques. Jaypee Brothers medical Publishers Ltd. Chapter 40; pp. 299-307.
10. Arya SA, Dalal S, **Brewster LP**. Hemodialysis Access in North America. IN: Dardik, Farber; eds. Vascular Surgery A Global Perspective. Springer. Chapter 38; pp. 251-262.
11. Dalal S, **Brewster LP.** Ethics in Laboratory Research. IN: Success in Academic Research: Basic Science. Springer Nature, 2019.

d. Manuals, Videos, Computer Programs, and Other Teaching Aids:

1. **LP Brewster**, EL Chaikof, J Sarmiento. (Video) Resection of a symptomatic portal vein aneurysm in a patient with prior gastric bypass. Americas Hepato-Biliary Association 2011.
2. B Martin, **LP Brewster**. (SCORE curriculum). Surgical Ethics Curriculum: Competence. 2012
3. C Long, **LP Brewster**. (SCORE curriculum). Procedures: Vascular Access Devices. 2013.
4. J Ruddy, **LP Brewster**. Women and PAD CME Program. American Heart Association. Presentation of PAD. 2015.

e. Other Publications:

1. **Brewster LP.** Greisler HP. 2008.Good Rum, Good Captain, Good Ship—How the consent for biomedical devices and implants can be transformed from an event to a process by the surgeon scientist. Journal of Long-Term Effects of Medical Implants 18(1):51.

2. Moalem J**, Brewster LP,** James TA. 2009. Resident and Associates Society Statement on Further Work Hour Restriction. Bulletin of the American College of Surgeons. 94(1):19-21.

3. Powell AC, Nelson JS, Massarweh NN, **Brewster LP**, Santry HP. 2009. The Modern Surgical Lifestyle. Bulletin of the American College of Surgeons. 94(6):31-37.

4. Kasirajan K, **Brewster LP.** 2009. The Gore Flow Reversal System—the Emory experience. Endovascular Today, Supplement November S26-30.

5. **Brewster LP**, Greisler HP. (Ethics) 2010.There may be nothing new under the sun, but this is ridiculous. Journal of Vascular Surgery; 52:1697-1698.

6. **Brewster LP**, Glass C, De Martino RR, Hall H, Kauvar DS, Kokkosis A, Kreishman P, Boros M, Kalata E, Farber A. The Formal Response of Vascular Surgery Trainees to ACGME Recommendations. Submitted to ACGME. Epublished at: http://www.vascularweb.org/about/positionstatements/Documents/SVS%20Response%20to%20ACGME%20Proposed%20Standards.pdf

7. Rosado AM, **Brewster LP**. Regeneration: Letting the scaffold do the work. Commentary. Journal of Surgical Research; 2013;180(1): 49-50.

8. Allen R, **Brewster LP**. Gender Differences at the Cellular Level. Commentary. Journal of Surgical Research; 2013;180(1):45-6.

9. Wang R, **Brewster LP.** Gut to Artery to Ureter-Robbing Peter to Pay Paul. Commentary. Journal of Surgical Research; 2013;183(1):102-3.

10. Beal S, **Brewster LP.** Femoral and Carotid Intima Media Thickness—two different measurements in two different arteries. Commentary. Journal of Surgical Research; 2013;183(2);511-2.

11. Ramos C, **Brewster LP**. Advances, Pauses, and Future Opportunity for the Prevention of VTE in the Trauma Population. Commentary. JAMA Surgery. 2017;152(1):41.

2. References

a. Draper N, Smith H, *Applied Regression Analysis*, Second Edition, John Wiley & Sons: New York, NY, USA, 1981.

b. Martens H, Næs T. *Multivariate Calibration*, Second Edition. John Wiley & Sons: Chichester, UK, 1989.

c. Geladi, P, Kowalski, BR. "PLS Tutorial" *Anal. Chim. Acta.*, **185**(1), 1986.

d. PLS_Toolbox, Eigenvector Research, Inc., Manson, WA, USA.

e. Bro R, Kjeldahl, K, Smilde, AK, Kiers, Henk. "Cross-validation of component models: A critical look at current methods." *Analytical and bioanalytical chemistry*. 390. 1241-51 (2008). 10.1007/s00216-007-1790-1.

1. Cao A, Pandya AK, Serhatkulu GK, et al. A robust method for automated background subtraction of tissue fluorescence. *Jornal of Raman Spectroscopy.* 2007;38:1199-1205.

2. Minamikawa T, Harada Y, Koizumi N, et al. Label-free detection of peripheral nerve tissues against adjacent tissues by spontaneous Raman microspectroscopy. *Histochem Cell Biol.* 2013;139(1):181-193.

3. Morisaki S, Ota C, Matsuda K-i, et al. Application of Raman spectroscopy for visualizing biochemical changes during peripheral nerve injury in vitro and in vivo. *Journal of Biomedical Optics.* 2013;18(11):116011-116011.

4. Saxena T, Deng B, Stelzner D, Hasenwinkel J, Chaiken J. Raman spectroscopic investigation of spinal cord injury in a rat model. *J Biomed Opt.* 2011;16(2):027003.

5. Wang H, Ma F, Wang F, Liu D, Li X, Du S. Identification of motor and sensory fascicles in peripheral nerve trunk using immunohistochemistry and micro-Raman spectroscopy. *J Trauma.* 2011;71(5):1246-1251.

6. Huff TB, Cheng JX. In vivo coherent anti-Stokes Raman scattering imaging of sciatic nerve tissue. *J Microsc.* 2007;225(Pt 2):175-182.

7. Brewster L, Brey EM, Addis M, et al. Improving endothelial healing with novel chimeric mitogens. *Am J Surg.* 2006;192(5):589-593.

3. Abstract to VA Surgeons

4. NRMC Disclosure

Authors: Hirotomo Nakahara (1), Malcolm W. Upchurch (1), Olamide Alabi (1,2) Atef Salam (1,2), Luke Brewster (1,2, 3)
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Title: Walking is Rare and Hospitalization is common after Major Amputations

Introduction: Major lower extremity amputation (above and below knee [AKA, BKA]) is a significant event that is often the "last resort" in the vascular surgeons' arsenal. There are robust resources for Veterans to assist in fitting appropriate candidates with a prosthesis, but rehabilitation strategies to improve Veteran ambulation are lacking. This pilot study was designed to identify the time to healing and walking in the Veteran population that was expected to be able to ambulate after major amputation.

Methods: After approval by the institutional review board (IRB) at the Atlanta Veterans Administration Medical Center, Veterans who ambulated within one month prior to undergoing major lower extremity amputation were included into this prospective study. All of the participants had a desire towards future ambulation, were desirous of being fitted for a prosthetic towards that end, and were deemed able to meaningfully participate in rehabilitation. Patient comorbidities and outcomes were recorded and analyzed.

Results: After IRB approval was obtained, 28 Veterans were enrolled in this study.After IRB approval, 28 Veterans were consented and enrolled in this study. The patient's displayed standard comorbidities for this population, with 68% with diabetes mellitus and 40% with end stage renal disease requiring dialysis. Average follow up was 363 days at the end of which 20 or 28 Veterans were alive with a healed amputation wound. During this timeframe, 6 Veterans underwent revisions of their initial amputation and 5 Veterans died (3 of which were during the index hospitalization). Of the 25 Veterans discharged from the hospital, there was an average of 5 subsequent rehospitalizations during the follow-up period. Three month amputation wound healing rates were 44% (11/25). Ambulation post amputation rates at 3 months was 20% (5/25). The average time from operation to ambulation with a prosthesis was 7.4 months after operation.

Conclusions: Veterans at this particular institution who underwent major lower extremity amputation commonly required revision of their wounds and frequent return to hospital events, however, were not likely to achieve ambulation post amputation. For those that were able to ambulate with a prosthetic post major lower extremity amputation, they average time to achieve this was 7.4 months. Interestingly, this average time to walking with a prosthesis, while certainly prolonged, were similar between Veterans with early (3 months or less) wound healing and late (>3 months) wound healing. Ongoing efforts by our vascular surgery service are working to improve initial wound care to speed healing of wounds as well as redirect rehabilitation strategies in order to decrease the deconditioning that occurs in the time span from major amputation until prosthetic fitting.

Raman Spectroscopy and 3D Imaging as Decision Support Tools in the Assessment of Neuronal Fibrosis and Sarcopenia in Veterans and Combat Casualty Amputees

DoD FY14 OPORP Orthotics and Prosthetics Outcomes Research Award W81XWH-14-OPORP-OPORA

PI: Luke Brewster, Ph.D., M.D. **Org:** Atlanta VA Medical Center, AREF **Award Amount:** $434,910

Study/Product Aim(s)

Specific Aim 1: To build a predictive model for Veterans and Service Members that segregates those who are able to walk after major amputation from those who do not walk. The model will be developed through integration of objective markers such as volumetric muscle mass, degree of fibrosis in nerve and muscle in histology, and nerve fibrosis as measured by Raman spectroscopy.

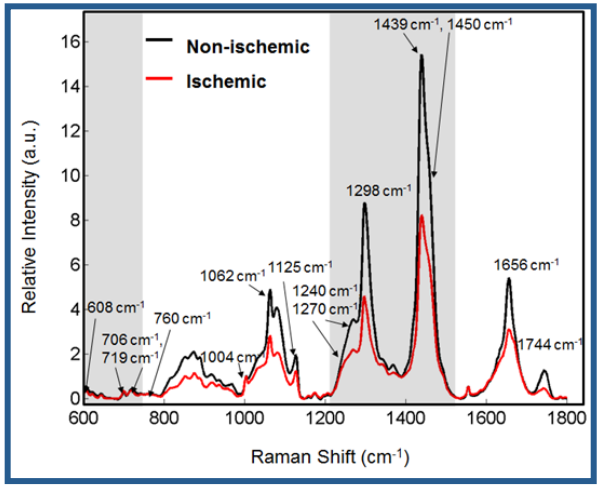
Specific Aim 2: To prospectively test the ability of the model developed in Specific Aim 1 to predict future ambulatory status in our Veteran and combat casualties undergoing major amputation.

Approach

Here we propose to build and then test a predictive model for ambulation after major amputation based on patient-specific pre- and peri-operative measurements.

Raman spectral comparison of sciatic and femoral nerves of peripheral nerves from ischemic and non-ischemic limbs.

Mean Raman metrics of control and ischemic nerves exhibit distinct differences. The deployment of *in vivo* Raman spectroscopy to **detect nerve injuries in patients**, including combat casualties, would offer the ability **to implement potential therapies for inhibiting nerve damage while in the operating room before functional evidence of neural deficit.**



Timeline and Cost

Activities FY	16	17
Data/samples for model calibration collected.		
Initial ambulation model developed.		
Data/samples for model validation collected.		
Text (Major aim/study/milestone)		
Estimated Budget ($435K)	**$240K**	**$196K**

Updated: (14Jun2019)

Goals/Milestones

FY16 Goal – Model Development

- ☐*HRPO approval.* **COMPLETE**
- ☐*20/49 patients enrolled at Atlanta VAMC (30 minimum for planned statistical testing); 0/2 patients enrolled at WRNMMC.*
- ☐*Specimens for model development collected and first analysis performed.* **COMPLETE**
- ☐*Predictive model built from calibration data.* **Model will be built at enrollment minimum of 30 patients**

FY17 Goal – Model Validation

- ☐*49 patients enrolled at Atlanta VAMC and 2 patients enrolled at WRNMMC.*
- ☐*Specimens for model validation collected.*
- ☐*Predictive model tested with validation data.*