



DEPARTMENT OF THE AIR FORCE  
59TH MEDICAL WING (AETC)  
JOINT BASE SAN ANTONIO - LACKLAND TEXAS

6 MAY 2016

MEMORANDUM FOR ST

ATTN: MAJ CHARLES ANTON FRIES

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled Enzyme Triggered Drug Delivery for Graft Targeted Immunosuppression and Neuroregeneration after VCA presented at/published to Association of Surgeons Great Britain and Ireland 11-13 May 2016 with MDWI 41-108, and has been assigned local file #16195.
2. Pertinent biographic information (name of author(s), title, etc.) has been entered into our computer file. Please advise us (by phone or mail) that your presentation was given. At that time, we will need the date (month, day and year) along with the location of your presentation. It is important to update this information so that we can provide quality support for you, your department, and the Medical Center commander. This information is used to document the scholarly activities of our professional staff and students, which is an essential component of Wilford Hall Ambulatory Surgical Center (WHASC) internship and residency programs.
3. Please know that if you are a Graduate Health Sciences Education student and your department has told you they cannot fund your publication, the 59th Clinical Research Division may pay for your basic journal publishing charges (to include costs for tables and black and white photos). We cannot pay for reprints. If you are 59 MDW staff member, we can forward your request for funds to the designated wing POC.
4. Congratulations, and thank you for your efforts and time. Your contributions are vital to the medical mission. We look forward to assisting you in your future publication/presentation efforts.

Linda Steel-Goodwin

LINDA STEEL-GOODWIN, Col, USAF, BSC  
Director, Clinical Investigations & Research Support

**PROCESSING OF PROFESSIONAL MEDICAL RESEARCH/TECHNICAL PUBLICATIONS/PRESENTATIONS**

**INSTRUCTIONS**

**USE ONLY THE MOST CURRENT 59 MDW FORM 3039 LOCATED ON AF E-PUBLISHING**

1. The author must complete page two of this form:
  - a. In Section 2, add the funding source for your study [ e.g., 59 MDW CRD Graduate Health Sciences Education (GHSE) (SG5 O&M); SG5 R&D; Tri-Service Nursing Research Program (TSNRP); Defense Medical Research & Development Program (DMRDP); NIH; Congressionally Directed Medical Research Program (CDMRP) ; Grants; etc.]
  - b. In Section 2, there may be funding available for journal costs, if your department is not paying for figures, tables or photographs for your publication. Please state "YES" or "NO" in Section 2 of the form, if you need publication funding support.
2. Print your name, rank/grade, sign and date the form in the author's signature block or use an electronic signature.
3. Attach a copy of the 59 MDW IRB or IACUC approval letter for the research related study. If this is a technical publication/presentation, state the type (e.g. case report, QA/QI study, program evaluation study, Informational report/briefing, etc.) in the "Protocol Title" box.
4. Attach a copy of your abstract, paper, poster and other supporting documentation.
5. Save and forward, via email, the processing form and all supporting documentation to your unit commander, program director or immediate supervisor for review/approval.
6. On page 2, have either your unit commander, program director or immediate supervisor:
  - a. Print their name, rank/grade, title; sign and date the form in the approving authority's signature block or use an electronic signature.
7. Submit your completed form and all supporting documentation to the CRD for processing (59crdpubspres@us.af.mil). If you have any questions or concerns, please contact the 59 CRD/ Publications and Presentations Section at 292-7141 for assistance.
8. The 59 CRD/Publications and Presentations Section will route the request form to clinical investigations, 502 ISG/JAC (Ethics Review) and Public Affairs (59 MDW/PA) for review and then forward you a final letter of approval or disapproval.
9. Once your manuscript, poster or presentation has been approved for a one-time public release, you may proceed with your publication or presentation submission activities, as stated on this form. **Note:** For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.
10. If your manuscript is accepted for scientific publication, please contact the 59 CRD/Publications and Presentations Section at 292-7141. This information is reported to the 59 MDW/CC. All medical research or technical information publications/presentations must be reported to the Defense Technical Information Center (DITC). See 59 MDWI 41-108, *Presentation and Publication of Medical and Technical Papers*, for additional information.

**NOTE:** All abstracts, papers, posters, etc., should contain the following disclaimer statement:

*"The views expressed are those of the [author(s)] [presenter(s)] and do not reflect the official views or policy of the Department of Defense or its Components"*

**NOTE:** All abstracts, papers, posters, etc., should contain the following disclaimer statement for research involving humans:

*"The voluntary, fully informed consent of the subjects used in this research was obtained as required by 32 CFR 219 and DODI 3216.02\_AFI 40-402."*

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*"The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended."*

**PROCESSING OF PROFESSIONAL MEDICAL RESEARCH/TECHNICAL PUBLICATIONS/PRESENTATIONS**

1. TO: CLINICAL RESEARCH	2. FROM: (Author's Name, Rank, Grade, Office Symbol) Charles Anton Fries, O-4	3. GME/GHSE STUDENT: <input checked="" type="checkbox"/> YES <input type="checkbox"/> NO	4. PROTOCOL NUMBER: NAVY15-05
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5. PROTOCOL TITLE: (NOTE: For each new release of medical research or technical information as a publication/presentation, a new 59 MDW Form 3039 must be submitted for review and approval.)  
Forelimb Allo-Transplantation in Swine (Sus scrofa) Umbrella Protocol for Optimization of Reconstruction of Battlefield Injuries Using the A

6. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED:  
Enzyme Triggered Drug Delivery for Graft Targeted Immunosuppression and Neuroregeneration after VCA

7. FUNDING RECEIVED FOR THIS STUDY?  YES  NO FUNDING SOURCE: AFMSA/59 MDW ST

8. DO YOU NEED FUNDING SUPPORT FOR PUBLICATION PURPOSES:  YES  NO

9. IS THIS MATERIAL CLASSIFIED?  YES  NO

10. IS THIS MATERIAL SUBJECT TO ANY LEGAL RESTRICTIONS FOR PUBLICATION OR PRESENTATION THROUGH A COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA), MATERIAL TRANSFER AGREEMENT (MTA), INTELLECTUAL PROPERTY RIGHTS AGREEMENT ETC.?  YES  NO NOTE: If the answer is YES then attach a copy of the Agreement to the Publications/Presentations Request Form.

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11a. PUBLICATION/JOURNAL (List intended publication/journal.)

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11c. POSTER (To be demonstrated at meeting: name of meeting, city, state, and date of meeting.)

11d. PLATFORM PRESENTATION (At civilian institutions: name of meeting, state, and date of meeting.)  
Association of Surgeons of Great Britain and Ireland/ 11-13 May 2016

11e. OTHER (Describe: name of meeting, city, state, and date of meeting.)



12. EXPECTED DATE WHEN YOU WILL NEED THE CRD TO SUBMIT YOUR CLEARED PRESENTATION/PUBLICATION TO DTIC  
NOTE: All publications/presentations are required to be placed in the Defense Technical Information Center (DTIC).

DATE  
May 10, 2016

13. 59 MDW PRIMARY POINT OF CONTACT (Last Name, First Name, M.I., email) Corpus Raul S, raul.s.corpus.ctr@mail.mil	14. DUTY PHONE/PAGER NUMBER 2105394404
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15. AUTHORSHIP AND CO-AUTHOR(S) List in the order they will appear in the manuscript.			
LAST NAME, FIRST NAME AND M.I.	GRADE/RANK	SQUADRON/GROUP/OFFICE SYMBOL	INSTITUTION (If not 59 MDW)
a. Primary/Corresponding Author Charles Anton Fries	O-4	59MDW ST RESTOR	
b. Sharon Lawson	CTR	59MDW ST RESTOR	
c. Lin Wang	O-3	59MDW ST RESTOR	
d. Renford Cindass	O-3	59MDW ST RESTOR	
e. Kevin Wu	CTR	59MDW ST RESTOR	
f. Michael R Davis	O-5	59MDW ST RESTOR	

I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401 JP, AND 59 MDW 41-108. I HAVE READ THE FINAL VERSION OF THE ATTACHED MATERIAL AND CERTIFY THAT IT IS AN ACCURATE MANUSCRIPT FOR PUBLICATION AND/OR PRESENTATION.

16. AUTHOR'S PRINTED NAME, RANK, GRADE Charles Anton Fries, O-4	17. AUTHOR'S SIGNATURE 	18. DATE April 26, 2016
19. APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE Michael R Davis, Lt Col, Director-RESTOR, Deputy Commander	20. APPROVING AUTHORITY'S SIGNATURE 	21. DATE April 26, 2016

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TO: Clinical Research Division 59 MDW/CRD Contact 292-7141 for email instructions.	22. DATE RECEIVED <b>4/26/2016</b>	23. ASSIGNED PROCESSING REQUEST FILE NUMBER <b>16195</b>
24. DATE REVIEWED <b>29 Apr 2016</b>		25. DATE FORWARDED TO 502 ISG/JAC
26. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES: <input checked="" type="checkbox"/> NO <input type="checkbox"/> YES If yes, give date. _____ <input type="checkbox"/> N/A		
27. COMMENTS <input checked="" type="checkbox"/> APPROVED <input type="checkbox"/> DISAPPROVED  The presentation is approved. The presentation will require a legal ethics review since it will be given to a foreign audience.		
28. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER <b>Rocky Calcote, PhD, Clinical Res. Administrator</b>	29. REVIEWER SIGNATURE CALCOTE.ROCKY.D.1178245844 <small>Digitally signed by CALCOTE.ROCKY.D.1178245844 DN: cn=Rocky, ou=5. Government, ou=502 ISG/JAC, o=CALCOTE.ROCKY.D.1178245844 Date: 2016.04.29 07:07:50 -0500</small>	30. DATE

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34. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	35. REVIEWER SIGNATURE	36. DATE

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40. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER <b>Christopher Carwile, TSgt/E-6, NCOIC, PA</b>	41. REVIEWER SIGNATURE CARWILE.CHRISTOPHER.S TEWART.1280477229 <small>Digitally signed by CARWILE.CHRISTOPHER.S DN: cn=Carwile, ou=5. Government, ou=502 ISG/JAC, o=CARWILE.CHRISTOPHER.S Date: 2016.05.06 11:22:07 -0500</small>	42. DATE <b>6 May 2016</b>

**4th ENDORSEMENT (59 MDW/SGVU Use Only)**

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# **Enzyme Triggered Drug Delivery for Graft Targeted Immunosuppression and Neuroregeneration after VCA**

*CA Fries, SD Lawson, LC Wang, R Cindass, K Wu, VS  
Gorantla, N Desai, J Fisher, J Karp, S Little, MR Davis*

*Sharon Lawson, MD*

*Research Fellow, RESTOR™ Program,  
General Surgery Resident, UTHSCSA*

*RESTOR™ Program, 59th Medical Wing, JBSA Lackland AFB*





# Disclaimer

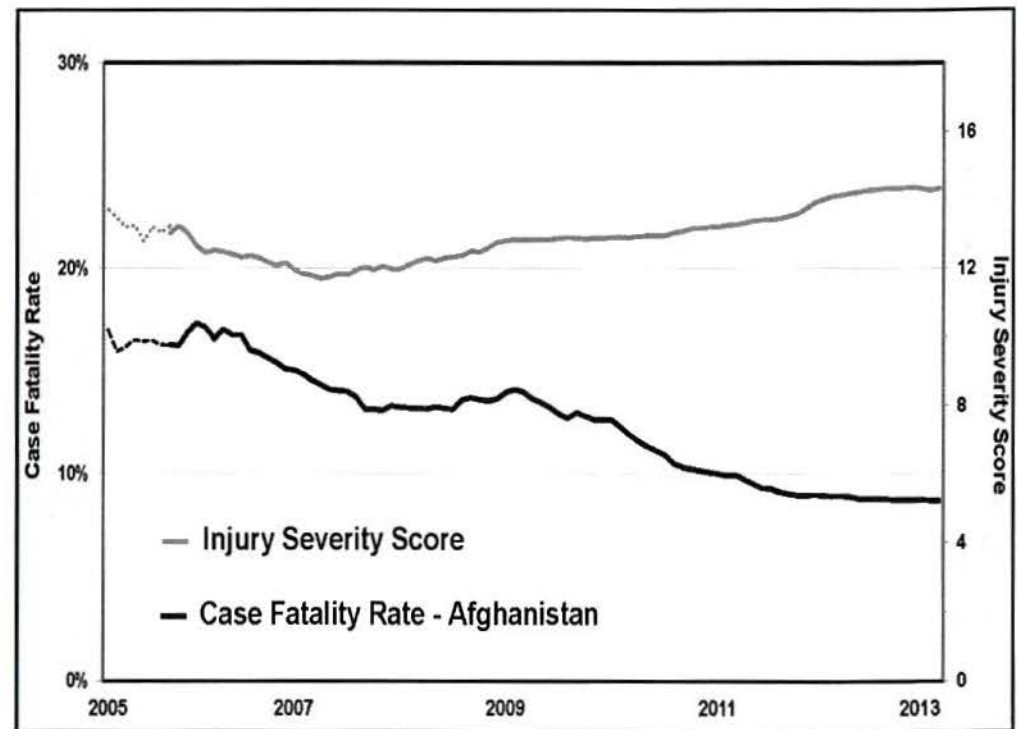
**The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense.**

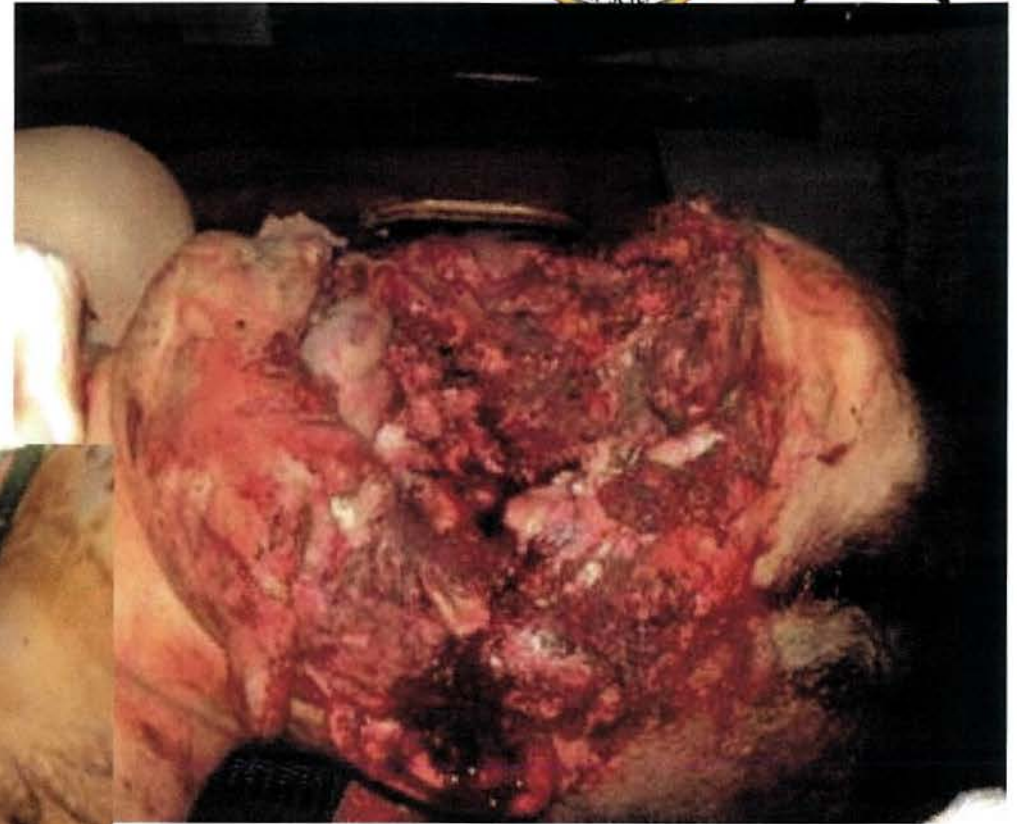
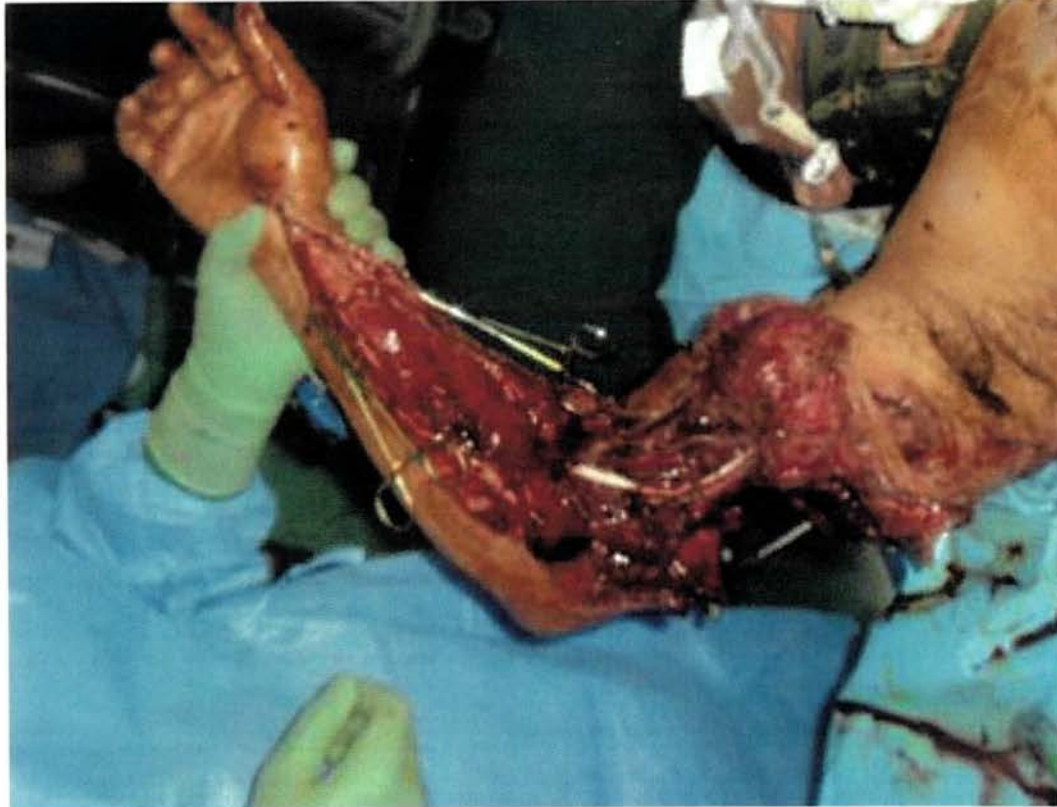
**The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended**



## Impact of Military Trauma Care and Research

- Improved combat casualty care
  - Increased injury severity score
  - Decreased case fatality rate

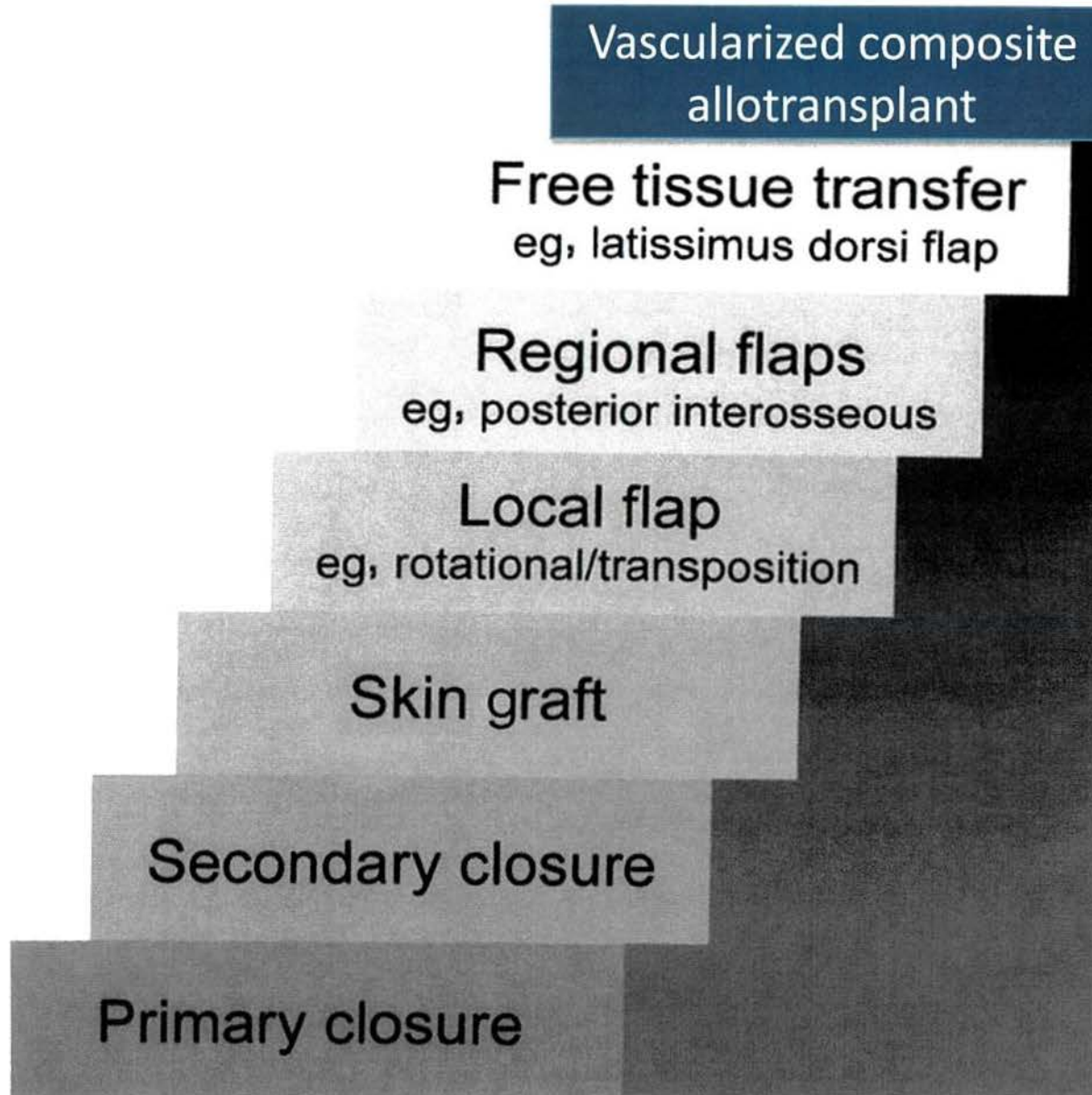








## New rung on the reconstructive ladder





## Vascularized Composite Allotransplantation (VCA)

- Current challenges and limitations
  - A life-enhancing but not a life-saving procedure
    - Requires lifelong systemic immunosuppression
      - Opportunistic infections: 88%
      - Metabolic complications: 70%
      - $\geq 1$  episode of acute rejection within 1<sup>st</sup> year: 85%
  - Requires highly motivated patients to comply with immunosuppression
  - Limited donor pool



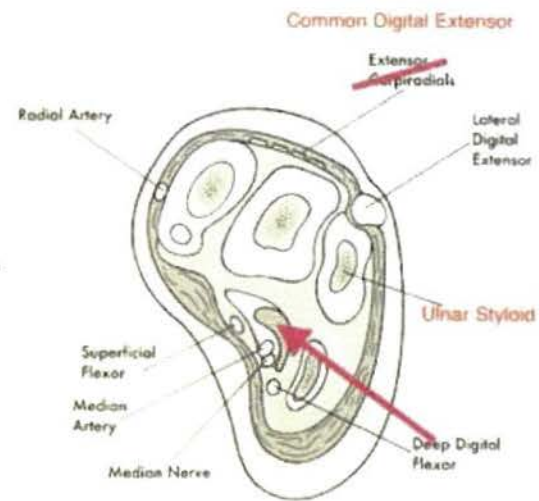
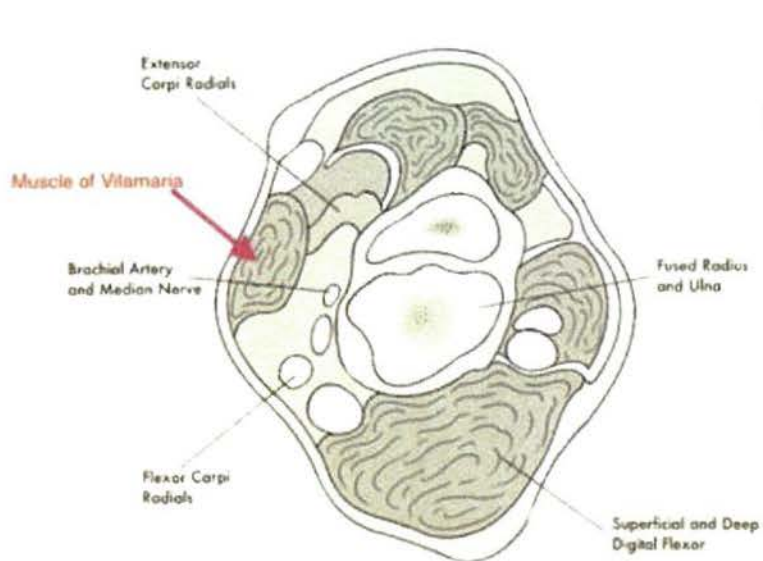
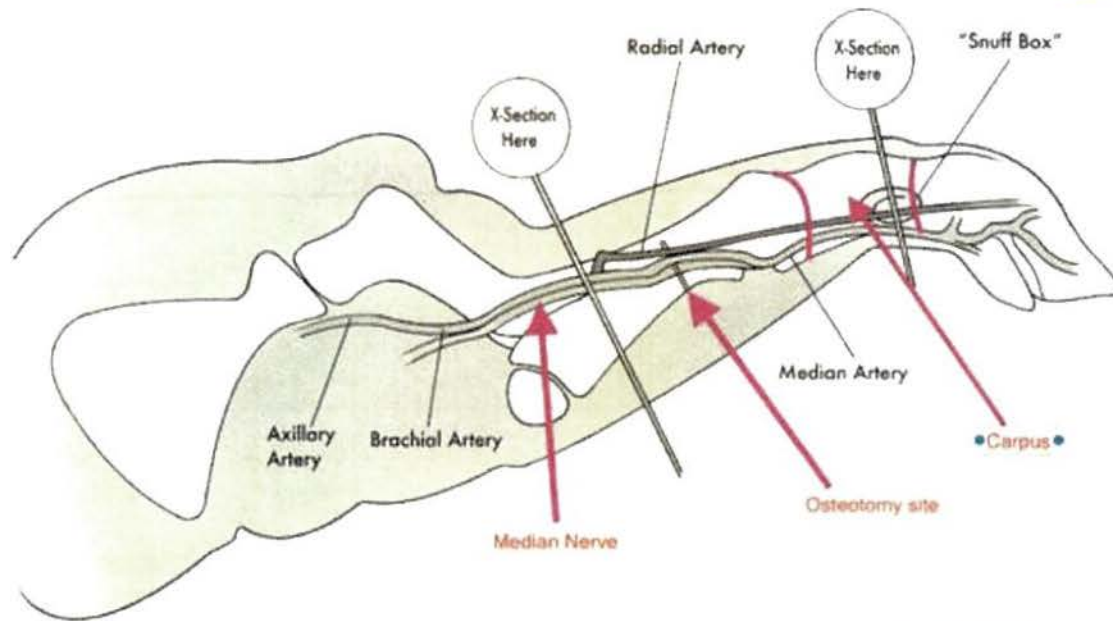
# Background



- Forelimb model of VCA
  - analogous to human hand transplant
- Evaluation of a drug eluting hydrogel for **localized** immunosuppression

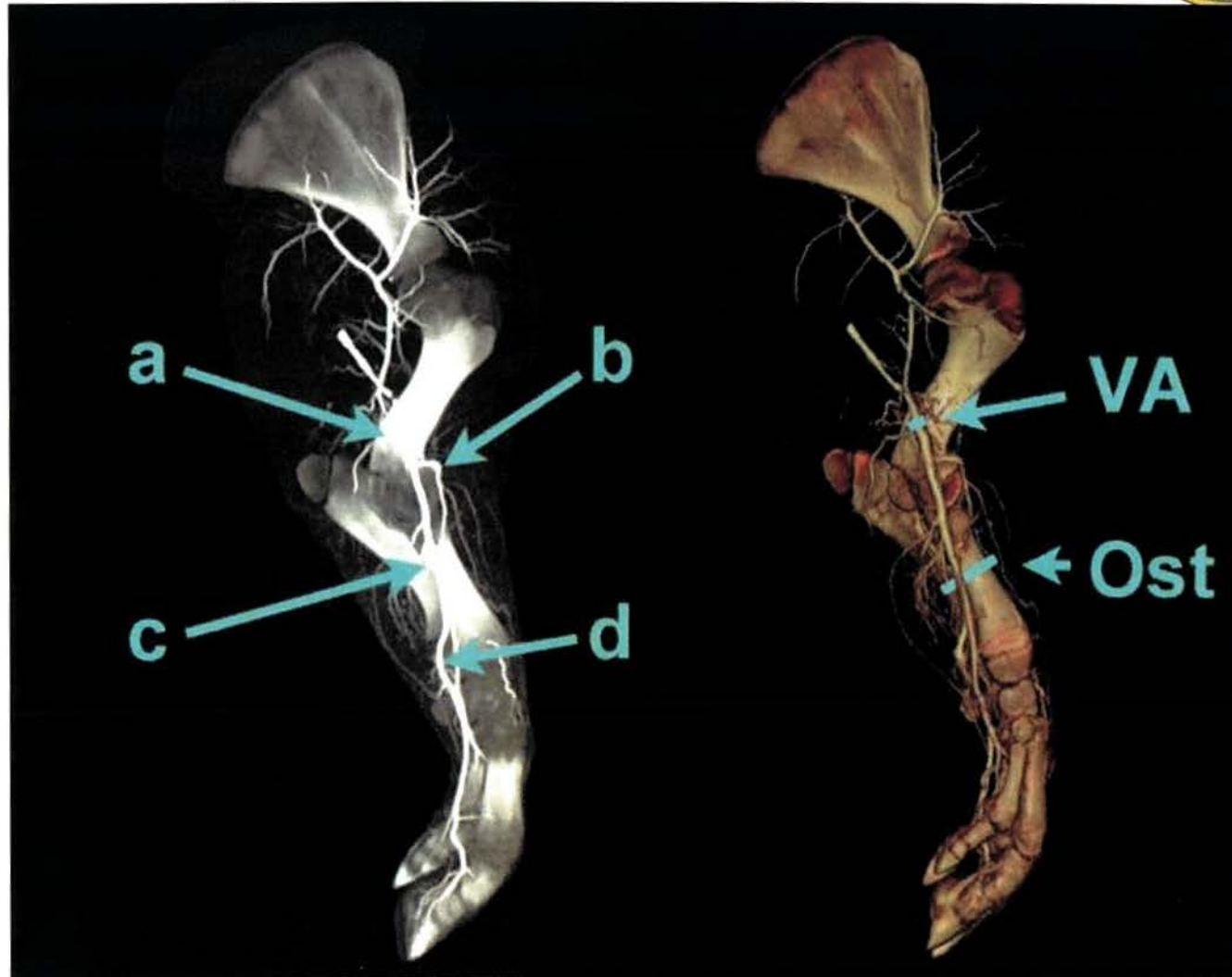


# Anatomy





# Orthotopic Forelimb Transplant



- A – Axillary Artery
- B – Radial Artery
- C – Interosseous Branch (of Fries)
- D – Median Artery)

- VA – Vascular Anastomosis
- Ost – Osteotomy Site





# Enzyme Activated Drug Eluting Hydrogel

RESEARCH ARTICLE

TRANSPLANTATION

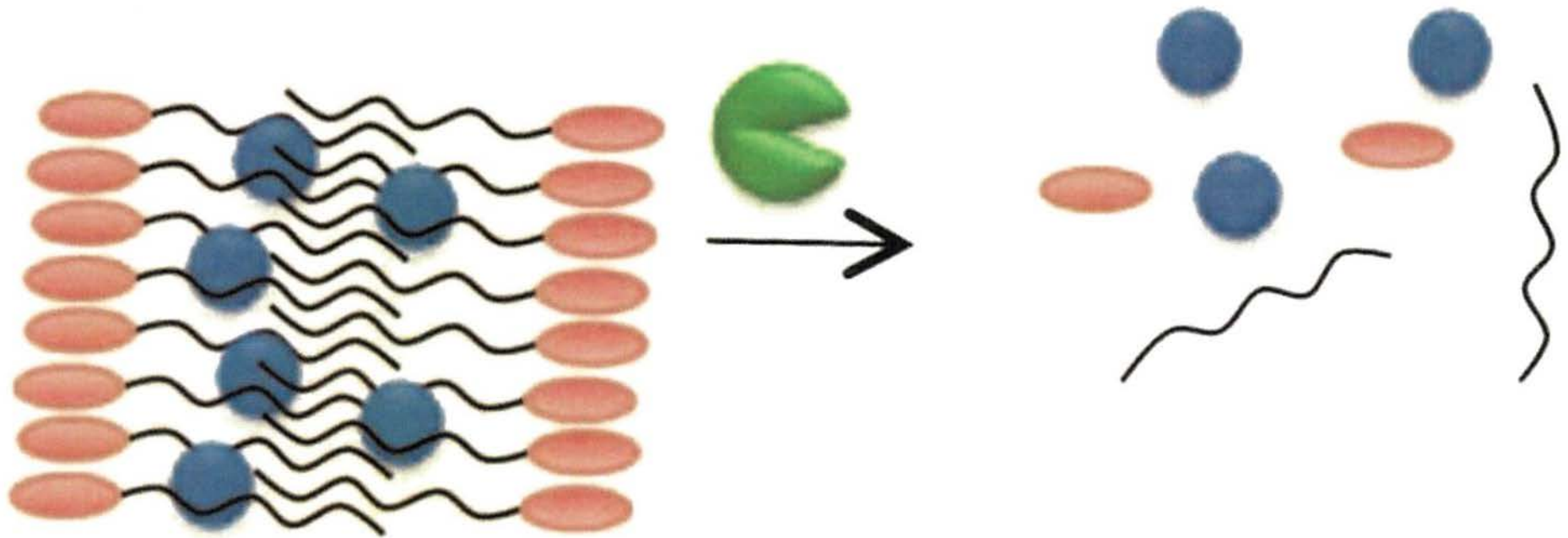
## A single localized dose of enzyme-responsive hydrogel improves long-term survival of a vascularized composite allograft

Thusitha Gajanayake,<sup>1,2\*</sup> Radu Olariu,<sup>1,2\*</sup> Franck M. Leclère,<sup>1,2</sup> Ashish Dhayani,<sup>3</sup> Zijiang Yang,<sup>4</sup> Anjan K. Bongoni,<sup>2,5</sup> Yara Banz,<sup>6</sup> Mihai A. Constantinescu,<sup>1,2</sup> Jeffrey M. Karp,<sup>4†</sup> Praveen Kumar Vemula,<sup>3†</sup> Robert Rieben,<sup>2†</sup> Esther Vögelin<sup>1,2</sup>

Currently, systemic immunosuppression is used in vascularized composite allotransplantation (VCA). This treatment has considerable side effects and reduces the quality of life of VCA recipients. We loaded the immunosuppressive drug tacrolimus into a self-assembled hydrogel, which releases the drug in response to proteolytic enzymes that are overexpressed during inflammation. A one-time local injection of the tacrolimus-laden hydrogel significantly prolonged graft survival in a Brown Norway-to-Lewis rat hindlimb transplantation model, leading to a median graft survival of >100 days compared to 33.5 days in tacrolimus only-treated recipients. Control groups with no treatment or hydrogel only showed a graft survival of 11 days. Histopathological evaluation, including anti-graft antibodies and complement C3, revealed significantly reduced immune responses in the tacrolimus-hydrogel group compared with tacrolimus only. In conclusion, a single-dose local injection of an enzyme-responsive tacrolimus-hydrogel is capable of preventing VCA rejection for >100 days in a rat model and may offer a new approach for immunosuppression in VCA.



# Enzyme Activated Drug Eluting Hydrogel







# Methods



- Three groups
  - Group 1: Controls – no immunosuppression
  - Group 2: High dose tacrolimus eluting hydrogel (81mg)
  - Group 3: Low dose tacrolimus eluting hydrogel (49mg)
- 1 swine leukocyte antigen (SLA) donor-recipient mismatch
- No systemic immunosuppression
- Hydrogel injected in the subcutaneous layer following revascularization
- AST, LDH, CK, TNF-a, IL-6, myoglobin, and biopsies were assessed for signs of systemic toxicity and/or acute rejection
- End-point – Banff grade 4 acute rejection or post-operative day 100



# BANFF Rejection scale



- **Grade 0**



- **Grade 4**





# Results

- Controls (2) – Grade IV Acute rejection mean POD 6
- Intervention (6) – Healed beyond POD 28 with no clinical or pathological rejection

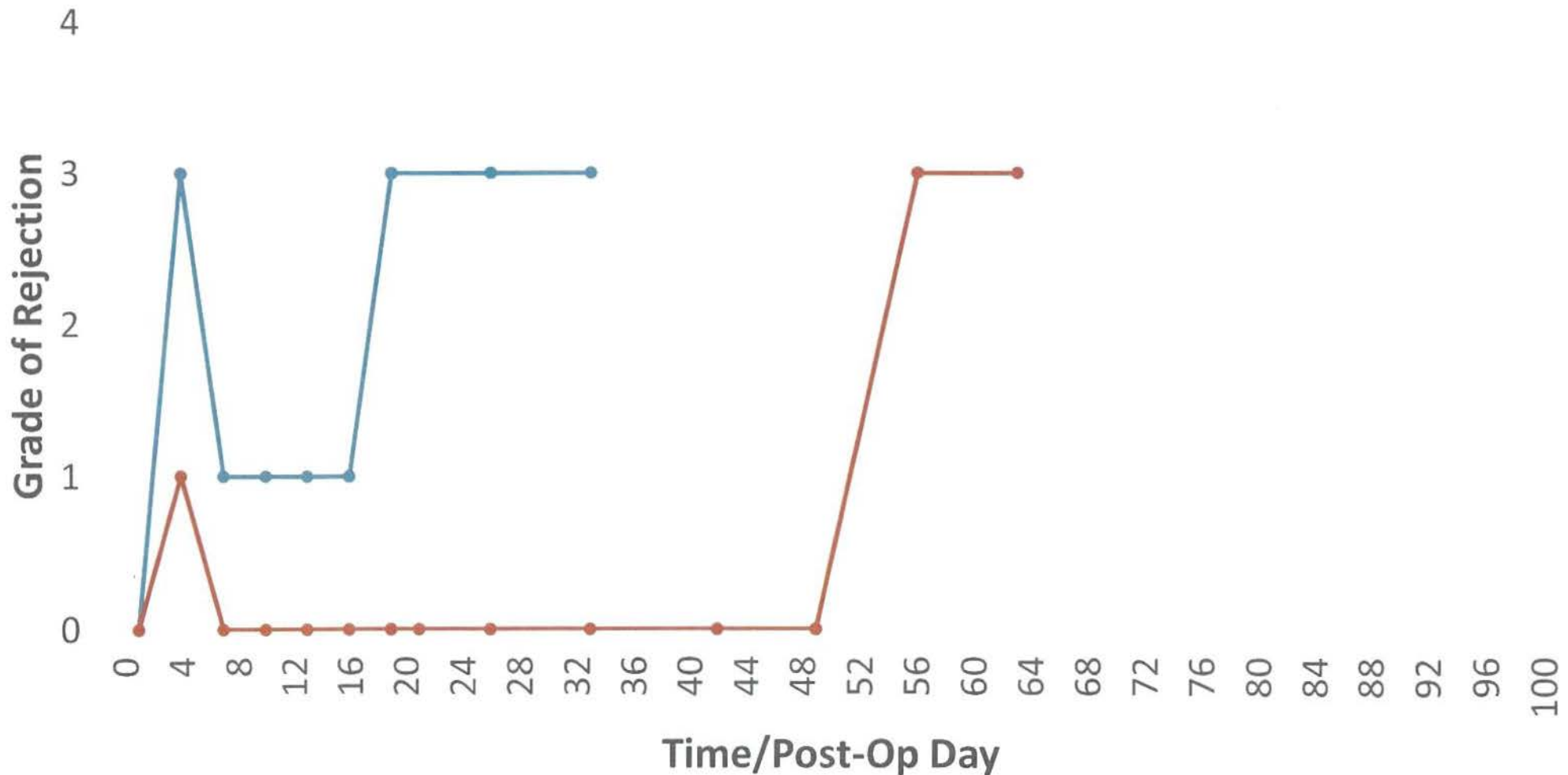




## Results: Rejection v. Time

### High Dose Tacrolimus

High Dose Tac 1    High Dose Tac 2

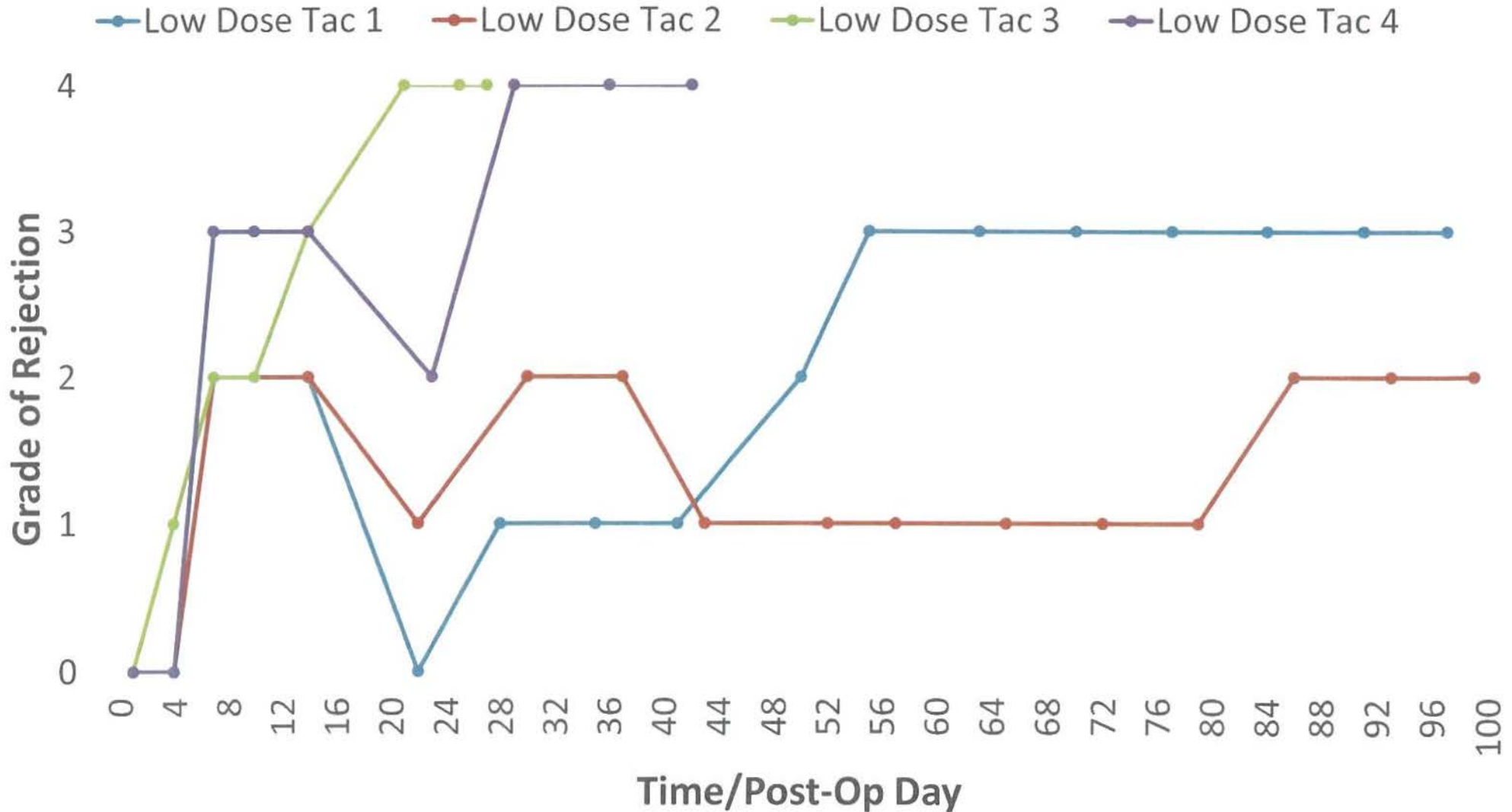




# Results: Rejection v. Time



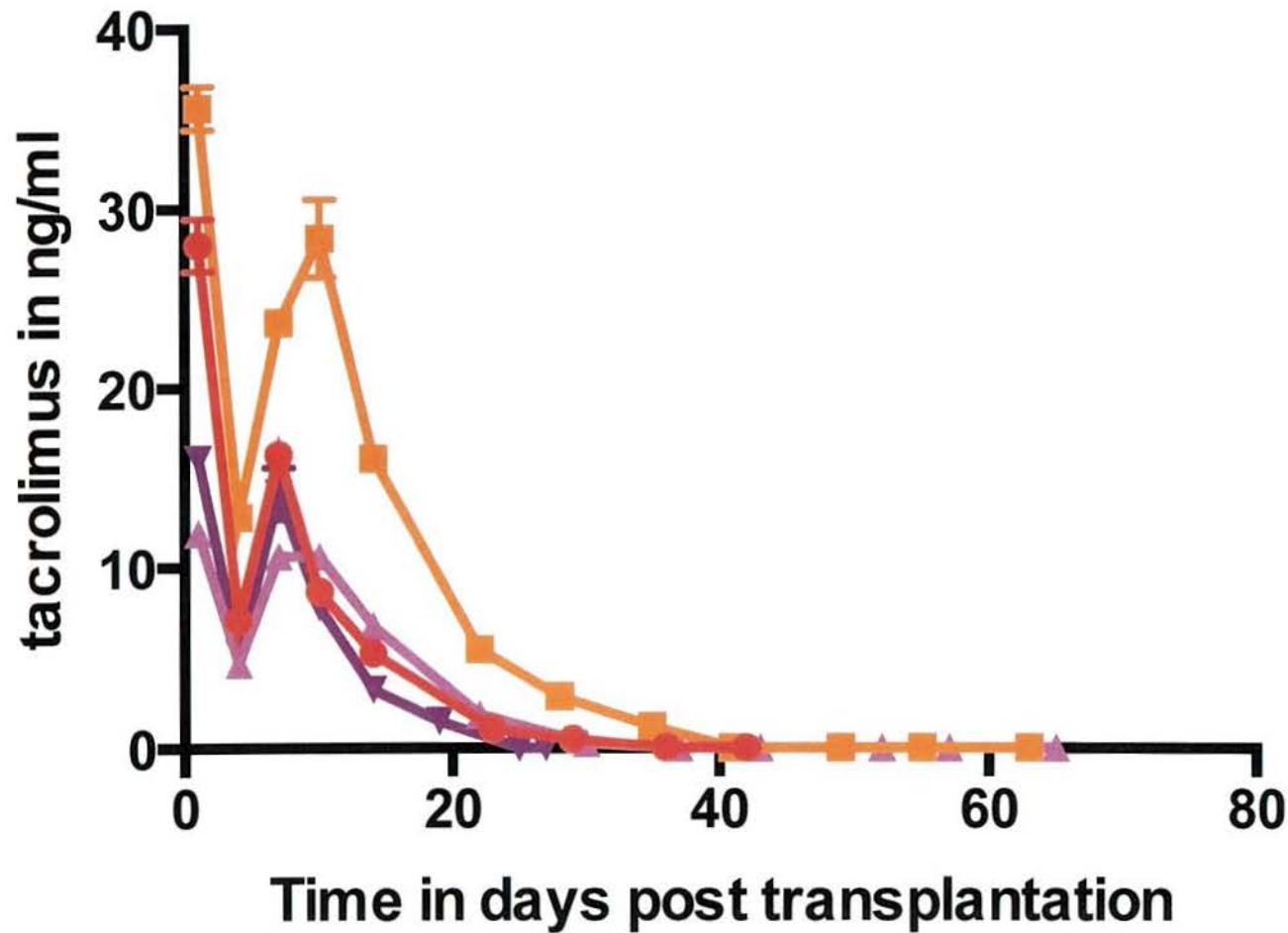
## Low Dose Tacrolimus





# Results

## Tacrolimus systemic levels

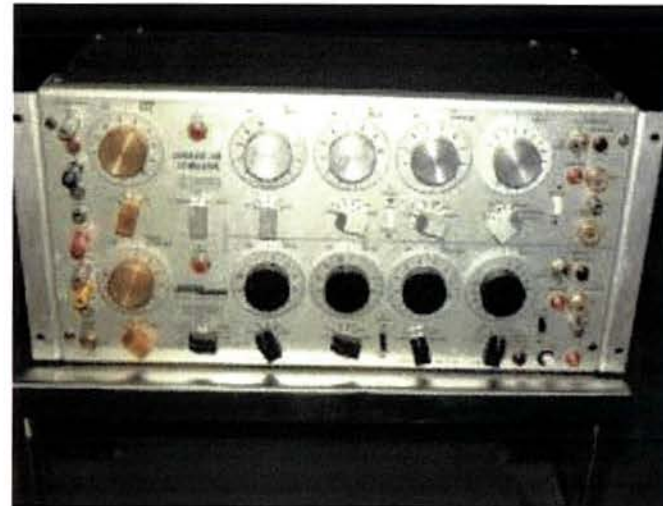




# Nerve Conduction Study



- Initial nerve conduction studies
  - Performed on two low dose swine from POD 57
    - Software/Equipment: Grass Technologies S88 Dual Output, Square Pulse Stimulator, National Instruments USB-6009, Data Acquisition Board, LABVIEW
  - Conduction velocity in mid-distal limb approximately 20 m/s (nl 50-70 m/s)





# Conclusions



- Graft embedded macrophage responsive hydrogels successfully achieved successful prevention of acute rejection in VCA.
  - prolongation of survival beyond 4 weeks
  - negligible / undetectable systemic tacrolimus levels
  - electrophysiologic evidence of nerve regeneration





# Future direction



- Future hydrogel protocols
  - Optimize dosing regimen
  - Analyze gel depletion and re-loading of the gel
  - Increase survival duration to evaluate longer term rejection and side effects profile



# Thank you



## **USAISR**

*Lt Col Michael Davis  
Lt Col Dmitry Tuder  
Dr Shari Lawson  
Dr Kevin Wu  
CPT Lin Wang  
Mr Raul Corpus*

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