DEPARTMENT OF THE AIR FORCE
59TH MEDICAL WING (AETC)
LACKLAND AIR FORCE BASE TEXAS

18 MAR 2016

MEMORANDUM FOR SGVT
ATTN: LT COL MICHAEL R DAVIS

FROM: 59 MDW/SGVU

SUBJECT: Professional Presentation Approval

1. Your paper, entitled **Locally Applied Enzyme Activated Tacrolimus Eluting Hydrogels Significantly Delay the Onset of Acute Rejection of VCA Grafts** presented at **2016 Society of Military Surgeons, Boston, MA, 17-19 March 2016** with MDWI 41-108, and has been assigned local file #**16139.**

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

1. TO: CLINICAL RESEARCH	2. FROM: (Author's Name, Rank, Grade, Office Symbol)	3. GME/GHSE STUDENT	4. PROTOCOL NUMBER
	Michael R Davis, O-6, Lt Col, 59MDW ST	☒ YES ☐ NO	Navy 15-09

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.)

Vascularized Composite Allotransplantation (VCA) in Swine (Sus scrofa) for Optimization of Reconstruction of Battlefield Injuries Using the

6. TITLE OF MATERIAL TO BE PUBLISHED OR PRESENTED:

Locally Applied enzyme activated tacrolimus eluting hydrogels significantly delay the onset of acute rejection of VCA grafts

7. FUNDING RECEIVED FOR THIS STUDY? ☒ YES ☐ NO FUNDING SOURCE: 59MDW 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.

11. MATERIAL IS FOR: ☒ DOMESTIC RELEASE ☐ FOREIGN RELEASE
CHECK APPROPRIATE BOX OR BOXES FOR APPROVAL WITH THIS REQUEST. ATTACH COPY OF MATERIAL TO BE PUBLISHED/PRESENTED.

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

☐ 11b. PUBLISHED ABSTRACT (List intended journal.)

☐ 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 meting.)
2016 Society of Military Surgeons, Boston MA 11-16 Mar 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	
March 18, 2016	

13. 59 MDW PRIMARY POINT OF CONTACT (Last Name, First Name, M.I., email)	14. DUTY PHONE/PAGER NUMBER
Corpus, Raul S raul.s.corpus.ctr@mail.mil	210.539.4404

15. AUTHORSHIP AND CO-AUTHOR(S) List in the order they will appear in the manuscript.

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d. Wang Lin C	O-3	59MDW ST	
e. Gorantla Vijay S	VOL	59MDW ST	
f. Davis Michael R	O-5	59MDW ST	

I CERTIFY ANY HUMAN OR ANIMAL RESEARCH RELATED STUDIES WERE APPROVED AND PERFORMED IN STRICT ACCORDANCE WITH 32 CFR 219, AFMAN 40-401_IP, AND 59 MDWI 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	17. AUTHOR'S SIGNATURE	18. DATE
Sharon Lawson VOL for		15 Mar 2016
19. APPROVING AUTHORITY'S PRINTED NAME, RANK, TITLE	**20. APPROVING AUTHORITY'S SIGNATURE**	**21. DATE**
Michael R. Davis, O-5, Director RESTOR, Deputy Commander, USA		15 Mar 2016

PROCESSING OF PROFESSIONAL MEDICAL RESEARCH/TECHNICAL PUBLICATIONS/PRESENTATIONS

1st ENDORSEMENT (59 MDW/SGVU Use Only)

TO: Clinical Research Division 59 MDW/CRD Contact 292-7141 for email instructions.	22. DATE RECEIVED 3/16/2016	23. ASSIGNED PROCESSING REQUEST FILE NUMBER 16-126
24. DATE REVIEWED		25. DATE FORWARDED TO 502 ISG/JAC

26. AUTHOR CONTACTED FOR RECOMMENDED OR NECESSARY CHANGES: [X] NO [ ] YES If yes, give date: _____ [ ] N/A

27. COMMENTS [X] APPROVED [ ] DISAPPROVED

- Title Slide lists CPT Cindass as Presenter while Form 3039 does not list him at all. Please correct Form 3039 or change title slide to reflect authorship listed on 3039.

28. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	29. REVIEWER SIGNATURE	30. DATE
Linda D Harris, GS-14 Chief, Ops Br	Linda D Harris	16 Mar 16

2nd ENDORSEMENT (502 ISG/JAC Use Only)

31. DATE RECEIVED	32. DATE FORWARDED TO 59 MDW/PA

33. COMMENTS [XX] APPROVED (In compliance with security and policy review directives.) [ ] DISAPPROVED

Slide presentation includes disclaimer required by the JointEthics Regulation. There are no ethics issues with making this presentation at the 2016 Society of Military Surgeons Meeting.

34. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	35. REVIEWER SIGNATURE	36. DATE
Holly J. Mackey, GS-13	Holly J Mackey	16 Mar 2016

3rd ENDORSEMENT (59 MDW/PA Use Only)

37. DATE RECEIVED	38. DATE FORWARDED TO 59 MDW/SGVU
17 March 2016	18 March 2016

39. COMMENTS [✓] APPROVED (In compliance with security and policy review directives.) [ ] DISAPPROVED

40. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	41. REVIEWER SIGNATURE	42. DATE
Christopher Carwile, TSgt/E-6, NCOIC, PA	CARWILE.CHRISTOPHER.STEWART.1280477229 Digitally signed by CARWILE.CHRISTOPHER.STEWART.1280477229 Date: 2016.03.18 11:08:21 -05'00'	18 March 2016

4th ENDORSEMENT (59 MDW/SGVU Use Only)

43. DATE RECEIVED	44. SENIOR AUTHOR NOTIFIED BY PHONE OF APPROVAL OR DISAPPROVAL [ ] YES [ ] NO [ ] COULD NOT BE REACHED [ ] LEFT MESSAGE

45. COMMENTS [ ] APPROVED [ ] DISAPPROVED

46. PRINTED NAME, RANK/GRADE, TITLE OF REVIEWER	47. REVIEWER SIGNATURE	48. DATE

Locally applied enzyme activated tacrolimus eluting hydrogels significantly delay the onset of acute rejection of VCA grafts

CPT Renford Cindass Jr., MD

* This person should be the Primary Author on Form 3039

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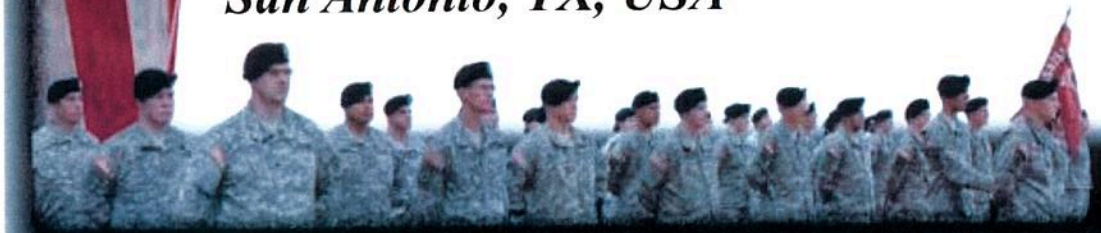
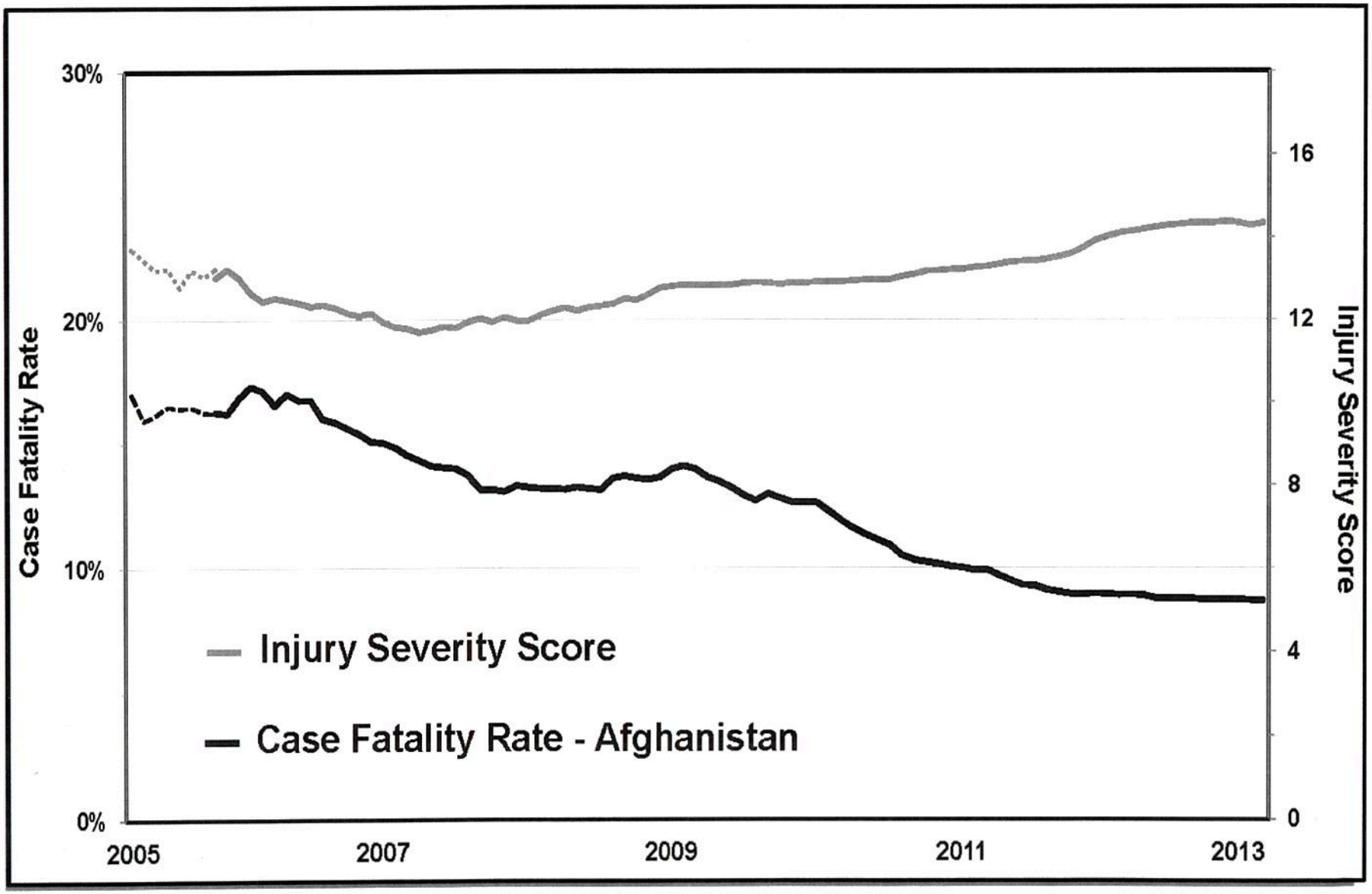


Disclaimer

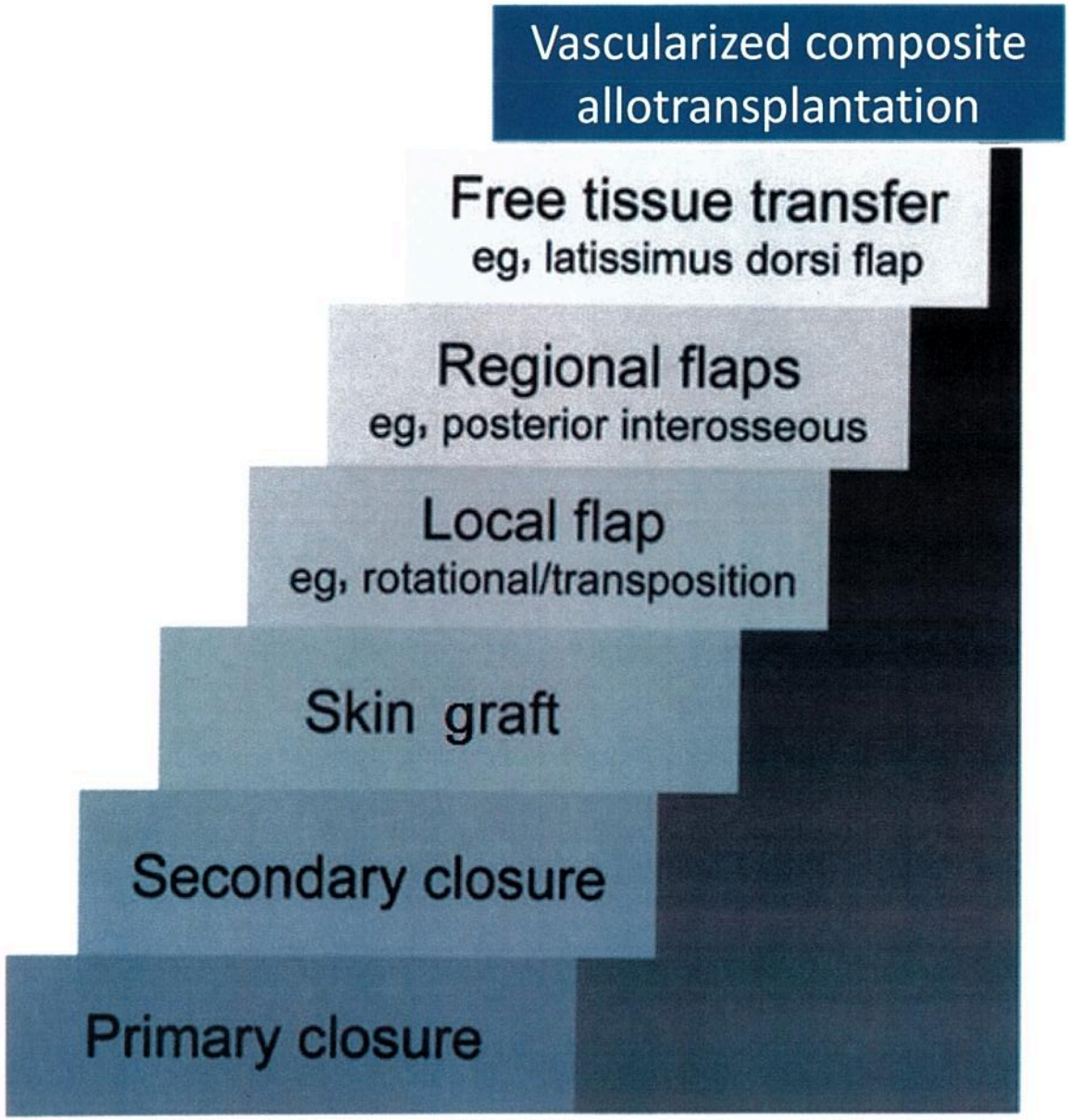
The opinions or assertions contained herein are the private views of the author and are not to be construed as official or as reflecting the views of the Department of Defense.

The experiments reported herein were conducted according to the principles set forth in the National Institute of Health Publication No. 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



New rung on the reconstructive ladder



Vascularized Composite Allotransplantation (VCA)

- Multiple types of tissues are transplanted as a single functional unit
- Replaces like with like and restores form and function
- Eliminates autologous donor site morbidity and minimizes the need for multiple reconstructive procedures

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%
      - ≥1 episode of acute rejection within 1st year: 85%
  - Limited to highly motivated patients
  - Limited donor pool

Murphy, B. D., *et al* (2013). Vascularized composite allotransplantation: An update on medical and surgical progress and remaining chanllenges. JPRAS, 66, 1449-1455.

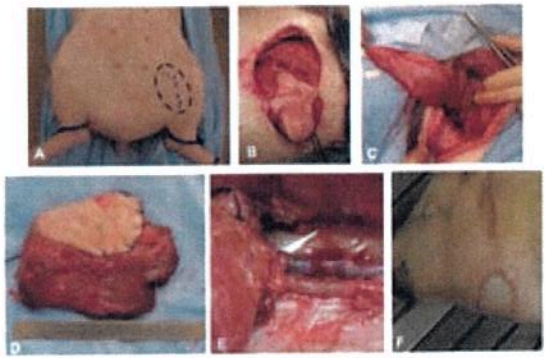
Background

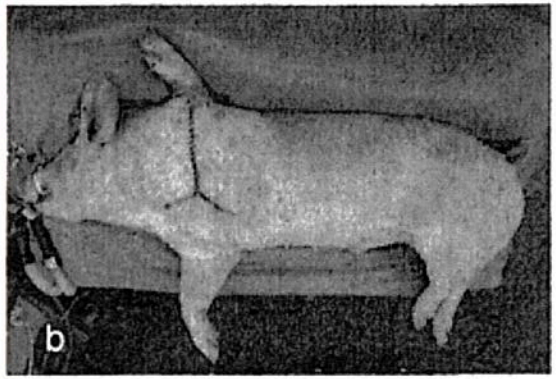
- A novel model of VCA

- Evaluation of a drug eluting hydrogel

A New Model

- Small animal models are technically challenging and lack immunologic maturity
- Previous orthotopic models in non-human primates and canines are no longer in use
- Currently only heterotopic swine models exist





Hettiaratchy, S., Melendy, E., Randolph, M. A., Coburn, R. C., Neville, D. M., Sachs, D. H., et al. (2004). Tolerance to composite tissue allografts across a major histocompatibility barrier in miniature swine. Transplantation, 77(4), 514–521.

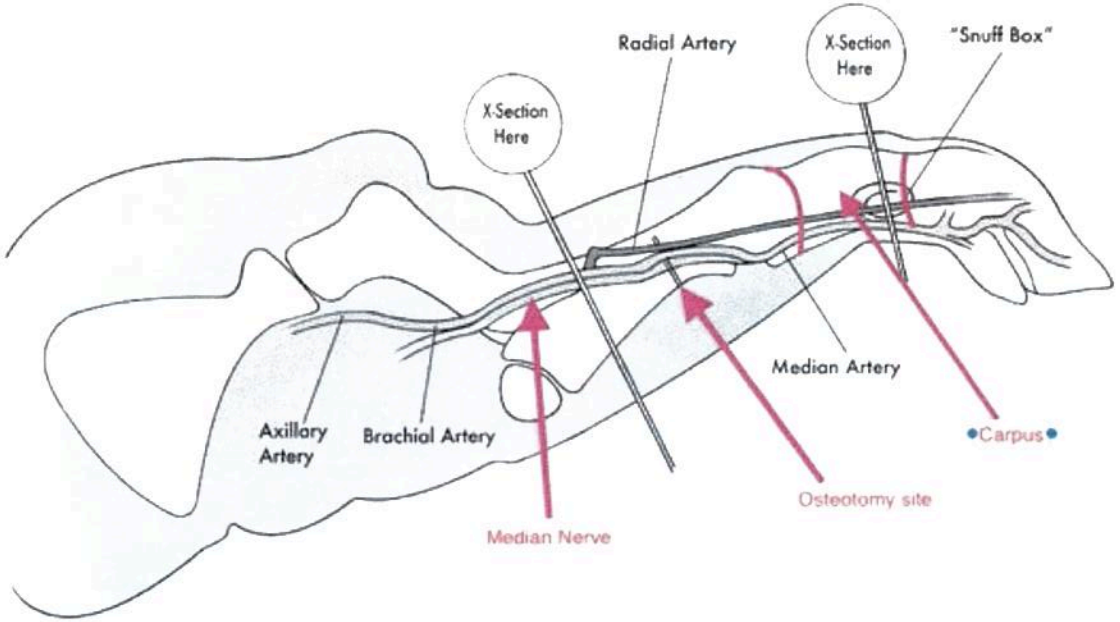
Leto Barone, A. A., Leonard, D. A., Torabi, R., Mallard, C., Glor, T., Scalea, J. R., et al. (2013). The gracilis myocutaneous free flap in swine: an advantageous preclinical model for vascularized composite allograft transplantation research. Microsurgery, 33(1), 51–55

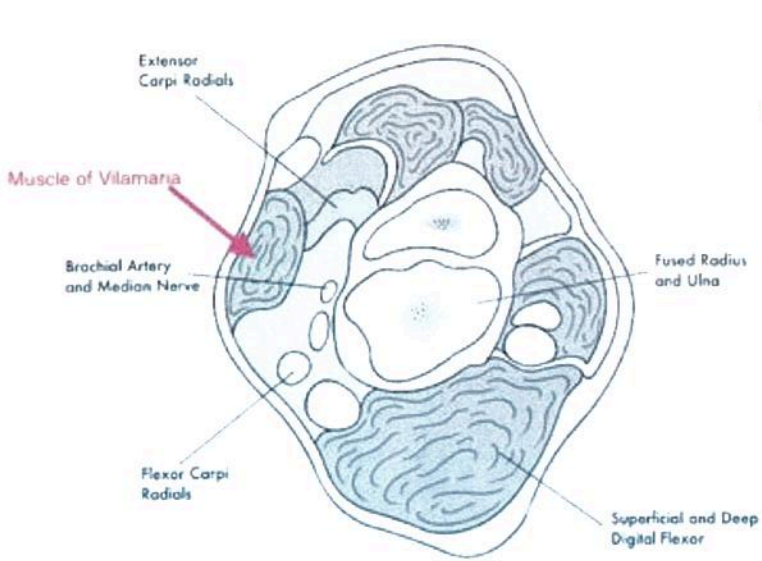
Kiermeir, D. M., Meoli, M., Müller, S., Abderhalden, S., Vögelin, E., & Constantinescu, M. A. (2013). Evaluation of a porcine whole-limb heterotopic autotransplantation model. Microsurgery, 33(2), 141–147

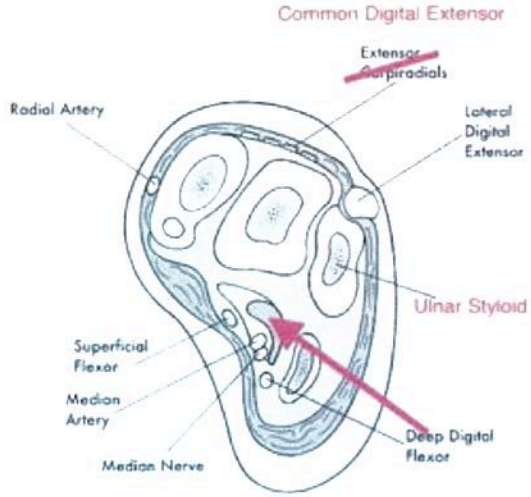
A New Model

- Ethically acceptable
- Reproducible
- Genetically controlled animals
- Orthotopic model to assess functionality
- Evaluation of bone, tendon and nerve healing

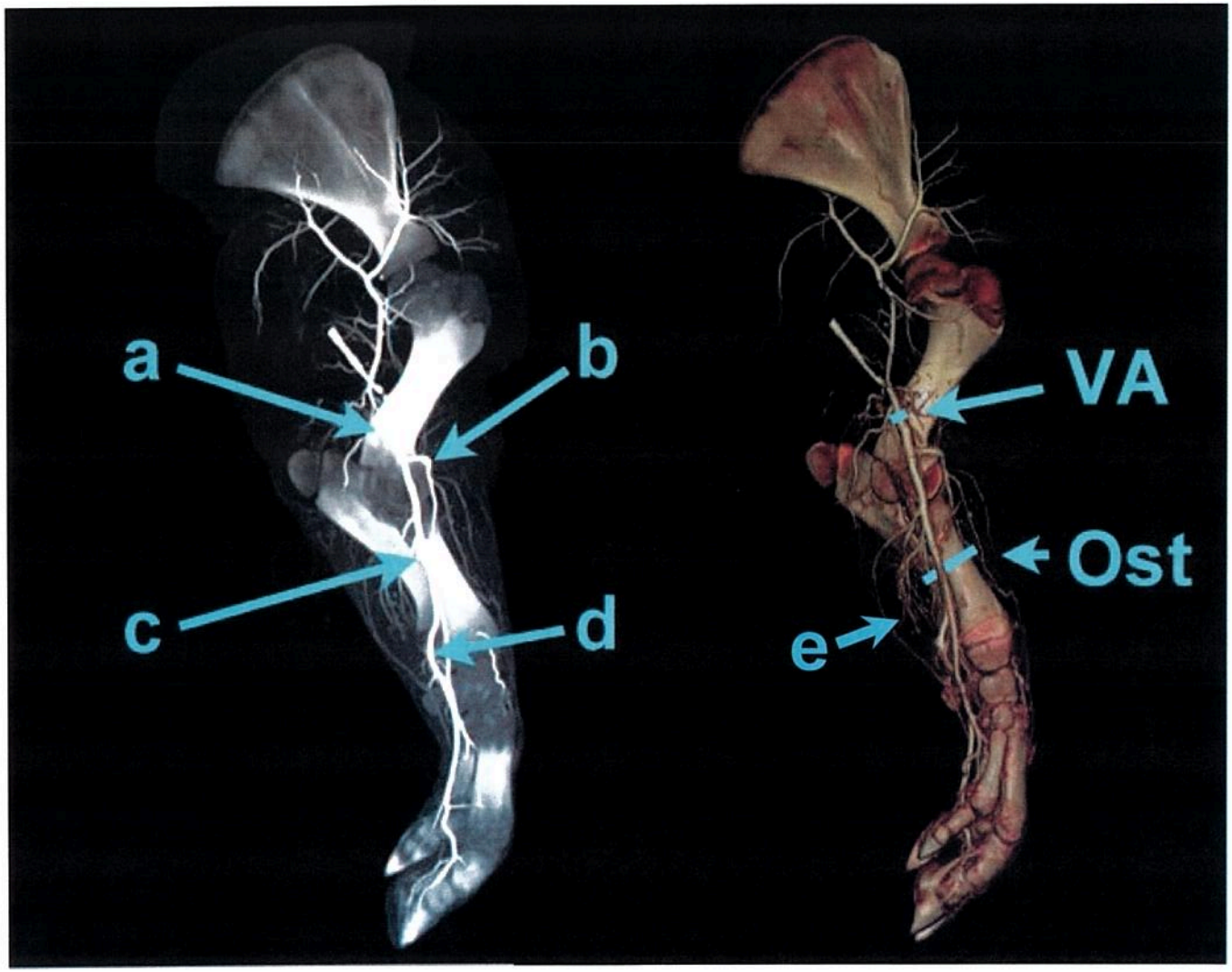
Anatomy


Radial Artery
X-Section Here
X-Section Here
"Snuff Box"
Median Artery
Axillary Artery
Brachial Artery
Carpus
Osteotomy site
Median Nerve


Extensor Carpi Radialis
Muscle of Vilamaria
Brachial Artery and Median Nerve
Fused Radius and Ulna
Flexor Carpi Radialis
Superficial and Deep Digital Flexor


Common Digital Extensor
Extensor Carpiradialis
Radial Artery
Lateral Digital Extensor
Ulnar Styloid
Superficial Flexor
Median Artery
Median Nerve
Deep Digital Flexor

Anatomy



A – Axillary Artery
B – Radial Artery
C – Interosseous Branch (of Fries)
D – Median Artery
E – The Nest (of Lawson)

VA – Vascular Anastomosis
Ost – Osteotomy Site

2 Weeks Post-op

Enzyme Activated Drug Eluting Hydrogel

RESEARCH ARTICLE

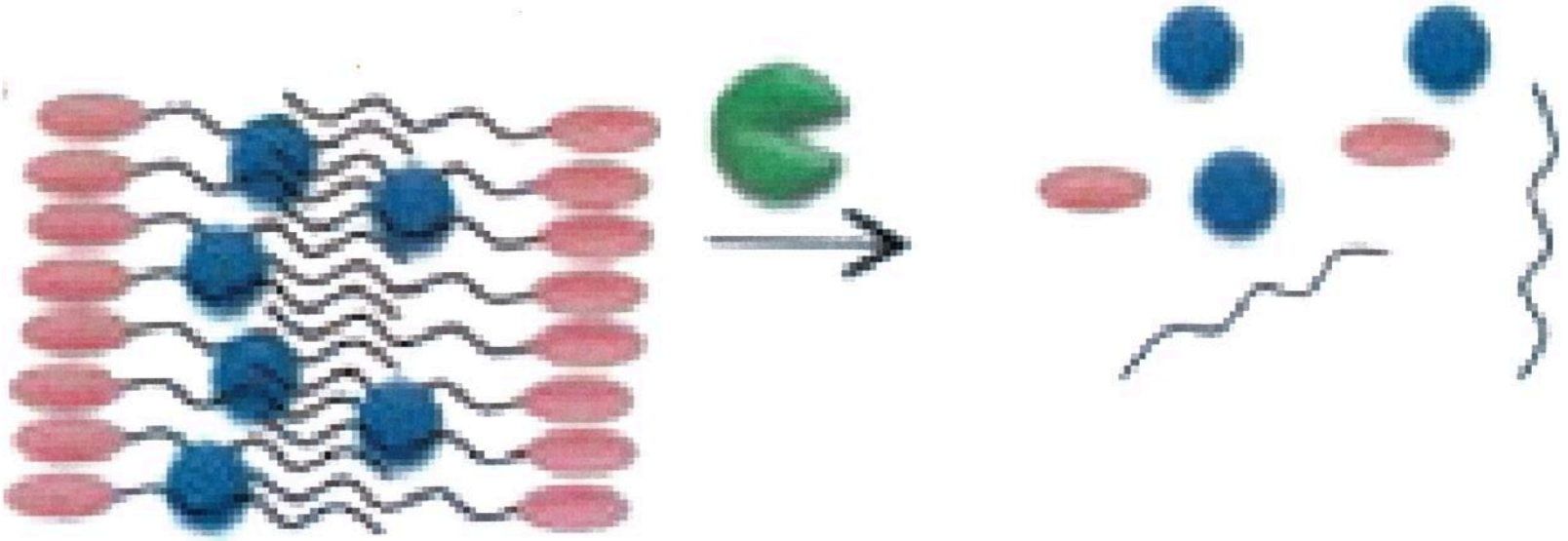
TRANSPLANTATION

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

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

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

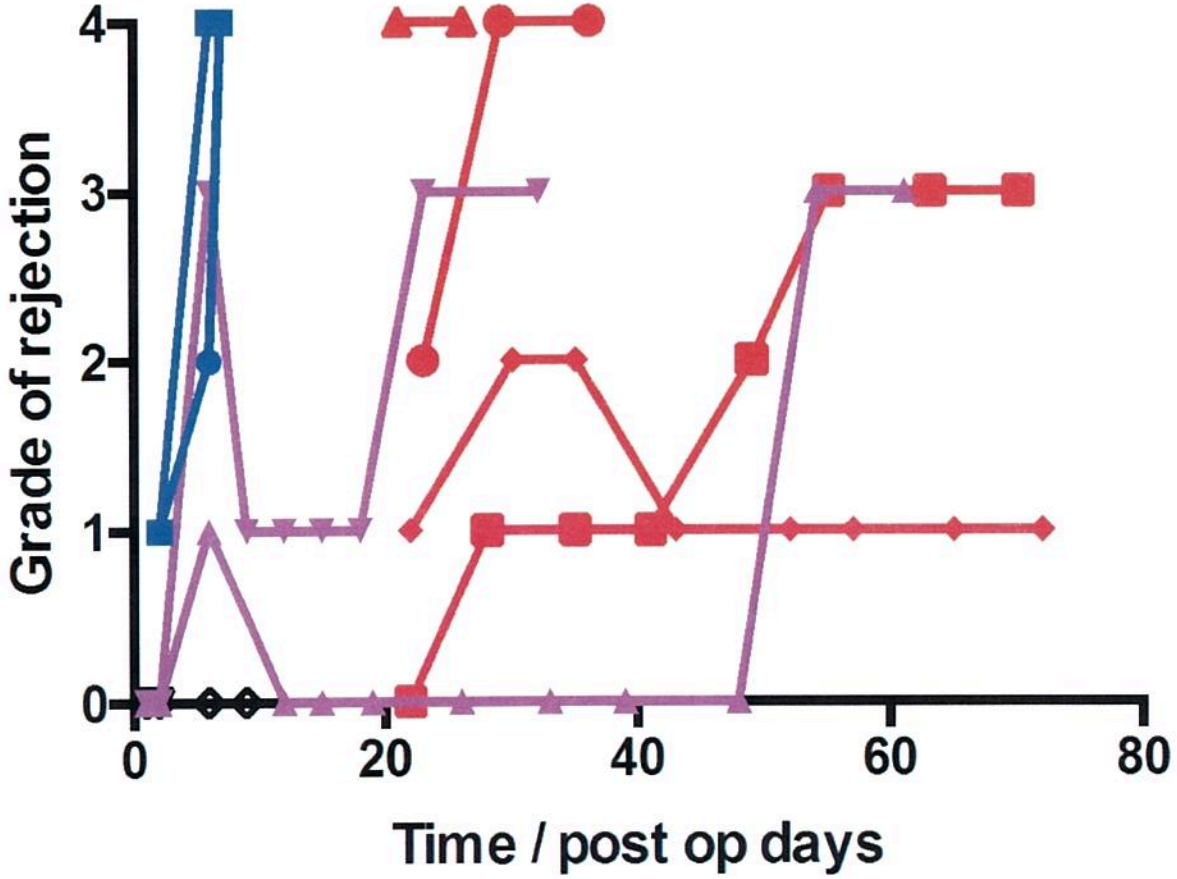


Methods

- Three groups
  - Group 1: Controls – no immunosuppression
  - Group 2: High dose tacrolimus eluting hydrogel (84mg)
  - 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 100 days

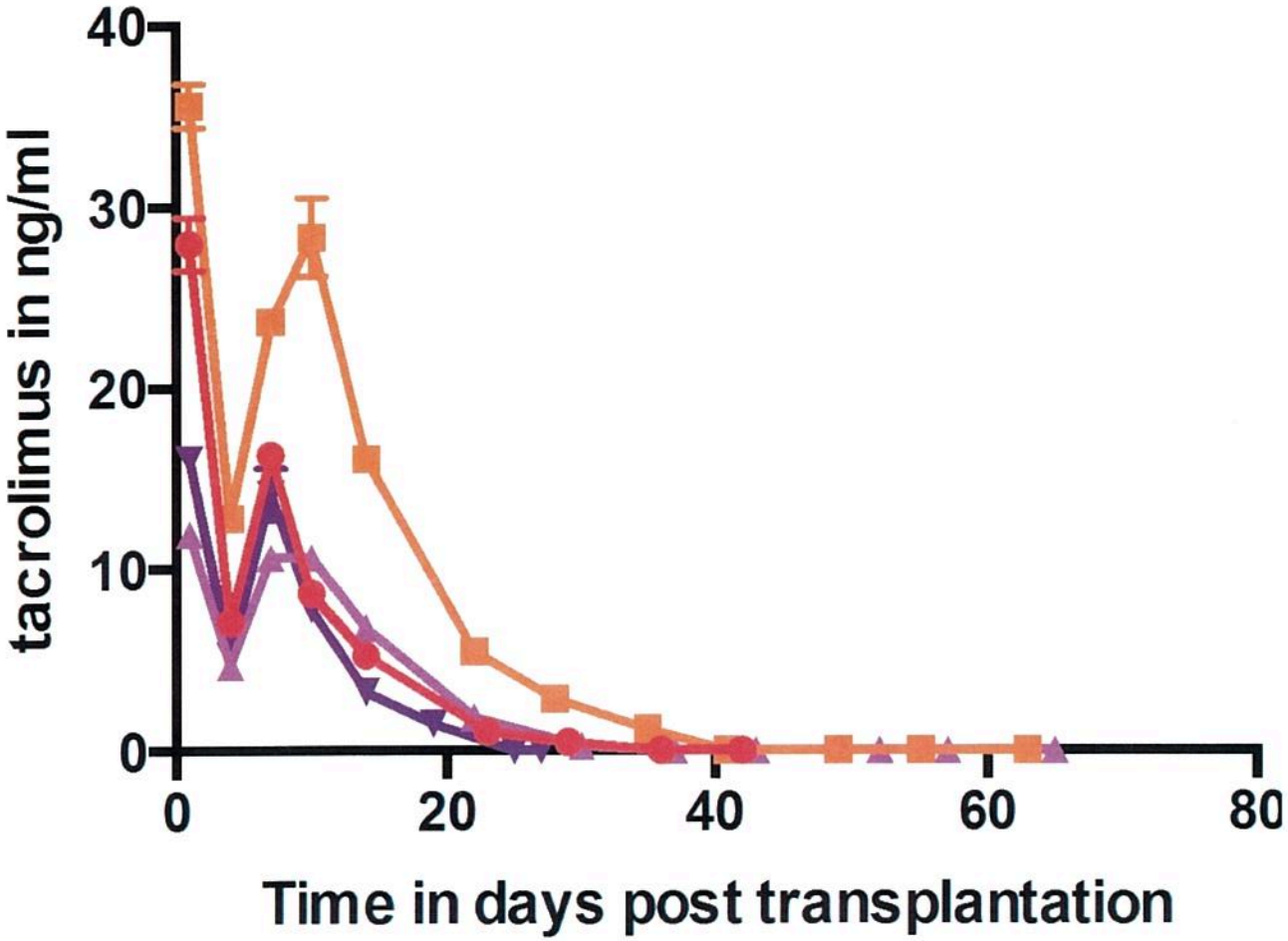
Results

Rejection against time of allographs



Results

Tacrolimus systemic levels



Conclusions

- The orthotopic model of swine VCA is an optimal model for investigating novel immunologic strategies
- Hydrogels are able to delay the onset of acute rejection with no gross safety concerns and without clinically detectable systemic levels of tacrolimus

Future direction

- Further hydrogel protocols to establish optimal dosing regimen and potential protocols for re-loading hydrogels

- Increased survival duration to evaluate longer term rejection and side effects profile

Thank you

USAISR/59MDW
Lt Col Michael Davis
Lt Col Dmitry Tuder
Dr Shari Lawson
Dr Kevin Wu
CPT Lin Wang
Mr Raul Corpus

Royal Centre for Defence Medicine
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