

AWARD NUMBER: W81XWH-15-1-0281

TITLE: Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Nonhuman Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol

PRINCIPAL INVESTIGATORS: Dr. Curtis L. Cetrulo

**CONTRACTING ORGANIZATION: Massachusetts General Hospital
Boston, MA 02114**

REPORT DATE: October 2016

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

DISTRIBUTION STATEMENT: Approved for public release; distribution is unlimited.

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. **PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.**

1. REPORT DATE October 2016		2. REPORT TYPE Annual		3. DATES COVERED 15 Sep 2015 - 14 Sep 2016	
4. TITLE AND SUBTITLE Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Nonhuman Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol				5a. CONTRACT NUMBER	
				5b. GRANT NUMBER W81XWH-15-1-0281	
				5c. PROGRAM ELEMENT NUMBER	
6. AUTHOR(S) Dr. Curtis L. Cetrulo E-Mail:ccetrulo@partners.org				5d. PROJECT NUMBER	
				5e. TASK NUMBER	
				5f. WORK UNIT NUMBER	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) AND ADDRESS(ES) Massachusetts General Hospital Boston, MA 02114				8. PERFORMING ORGANIZATION REPORT NUMBER	
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES) U.S. Army Medical Research and Materiel Command Fort Detrick, Maryland 21702-5012				10. SPONSOR/MONITOR'S ACRONYM(S)	
				11. SPONSOR/MONITOR'S REPORT NUMBER(S)	
12. DISTRIBUTION / AVAILABILITY STATEMENT Approved for Public Release; Distribution Unlimited					
13. SUPPLEMENTARY NOTES					
14. ABSTRACT The focus for Year 1 was to develop a polymeric local delivery system (PLDS) for tacrolimus (FK506), and characterize <i>in vitro</i> . The work included selecting and characterizing the polymer for the PLDS, optimizing a fabrication method, and developing characterization methods to test the pharmacokinetics after drug loading. To this end, the team synthesized and characterized a batch of the chosen tyrosine-derived polymer formulation, E1218(1k), and moved forward in finalizing the fabrication method for the PLDS. Method development for in-depth pharmacokinetic studies is currently underway, and preclinical animal studies are currently being planned for testing the PLDS in a small animal model as well as a non-human primate model.					
15. SUBJECT TERMS Drug delivery, immunosuppression, tacrolimus, FK506, vascularized composite allografts, immune rejection, preclinical, transplant, nonhuman primate model, degradable polymer, tyrosine-derived polycarbonate					
16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT Unclassified	b. ABSTRACT Unclassified	c. THIS PAGE Unclassified			USAMRMC
			Unclassified	14	19b. TELEPHONE NUMBER (include area code)

TABLE OF CONTENTS

	<u>Page No.</u>
1. Introduction	4
2. Keywords	4
3. Accomplishments	4
4. Impact	11
5. Changes/Problems	11
6. Products	12
7. Participants & Other Collaborating Organizations	13
8. Special Reporting Requirements	13

- 1. INTRODUCTION:** The purpose of this research is to develop an intraoperative, implantable, biomaterials-based, controlled release system for the local delivery of tacrolimus (a potent immunosuppressive drug) to prevent acute rejection episodes of vascularized composite allografts (VCAs) in non-human primates until delayed mixed chimerism can be established and subsequent withdrawal of immunosuppression can be safely performed. VCA provides the opportunity to restore complex anatomical and functional units, such as the hands or face, following devastating injury. These procedures have the potential to revolutionize the treatment of wounded warriors with extremity amputations and severe craniofacial injuries. However, minimization of immunosuppression and/or induction of tolerance represent the major impediment to widespread application of VCA procedures. In this work, a polymer-based device loaded with tacrolimus will be tested and analyzed using *in vitro* and *in vivo* techniques. *In vivo* models include a non-human primate model, which will be used for studies of VCA immunosuppression and development of tolerance induction protocols. These studies, if successfully completed, will enable us to initiate enabling studies required for a regulatory application.

- 2. KEYWORDS:** Drug delivery, immunosuppression, tacrolimus, FK506, vascularized composite allografts, immune rejection, preclinical, transplant, nonhuman primate model, degradable polymer, tyrosine-derived polycarbonate

- 3. ACCOMPLISHMENTS:**

Our overall objective is to demonstrate the *in vivo* safety and efficacy of the local site-specific administration of tacrolimus via an implantable polymeric local delivery system (PLDS) that will enable the reduction of systemic immunosuppression levels and avoid immunosuppression-related morbidity and rejection episodes that result in either graft loss or sensitization to donor bone marrow. The hypothesis is that the application of a PLDS for the controlled and sustained release of tacrolimus directly at the host-donor skin interface of the vascularized composite allotransplant (VCA) will prevent acute rejection episodes, reduce the need for systemic immunosuppression, and serve as a bridge to the induction of immunologic tolerance by delayed mixed chimerism. The project's specific aims are (1) to develop and characterize PLDS for tacrolimus and profile the *in vivo* release kinetics and local tissue distribution of tacrolimus in a small animal model; (2) optimize the adjunctive tacrolimus implant dose necessary for the reduction of systemic immunosuppression for upper extremity allotransplantation in a nonhuman primate (NHP) model; and (3) provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for upper extremity allotransplantation in an NHP model. The study design builds upon a substantial amount of preliminary data on the development of a controlled release system for the local delivery of calcineurin inhibitors (CNIs), and its completion will enable the initiation of investigational new drug-enabling studies with the belief that this technology will smoothly segue into clinical trials and be translated into a revolutionary immunosuppressive therapy directly beneficial to the success of currently ongoing clinical VCA tolerance protocol for hand transplantation.

What were the major goals of the project?

***Specific Aim 1:** To develop and characterize Polymeric Local Delivery Systems for tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model.*

***Specific Aim 2:** To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free facial allotransplantation in a non-human primate (NHP) model.*

***Specific Aim 3:** To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for facial allotransplantation in a non-human primate model.*

Tasks	Timeline (months)	Comments
Task 1. To develop and characterize Polymeric Local Delivery Systems for Tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model	1-18	
1.1. Formulate polymeric local delivery systems (PLDS) for tacrolimus and characterize in vitro.	1-11	
1.1.1. Polymer synthesis and characterizations	1	100% complete
1.1.2. Investigate thermal stability of tacrolimus in the presence of polymer	2	Ongoing and in progress.
1.1.3. Fabricate tacrolimus-loaded polymeric local delivery system (tacrolimus-PLDS) devices	2	50% complete – Preliminary devices approved by collaborator. Optimization in progress.
1.1.4. Investigate the irradiation stability of tacrolimus-PLDS	3	Delayed due to subaward execution delay
1.1.5. Perform in vitro polymer degradation and release study and assess bioactivity of released tacrolimus	4-11	Instrument methods currently under development. Release study pending final device size specifications.
1.2. Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS in a small animal model.	1-12	
1.2.1. Obtain IACUC and ACURO approval of animal subcutaneous implantation protocol	1-3	100% complete

1.2.2. Cohort 1 - Perform an exploratory study to obtain short-term, preliminary data on the in vivo release of tacrolimus-PLDS (15wt.%) and determine the maximum distance that tacrolimus diffuses away from the center of the device (Fabrication of devices + 1 month animals in life + analyses).	5-7	Delayed due to subaward execution delay
1.2.3. Cohort 2 - Optimize tacrolimus loading in the PLDS to provide a local tissue concentration of 4 ng/g/day or 0.4 ng/g/day (2 month study). (Fabrication of devices + 2 month animals in life + analyses).	8-12	Delayed (dependent upon completion of 1.2.2).
1.2.3. Cohort 3 - Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS for 4 months.	13-18	Delayed (dependent upon completion of 1.2.2 and 1.2.3).

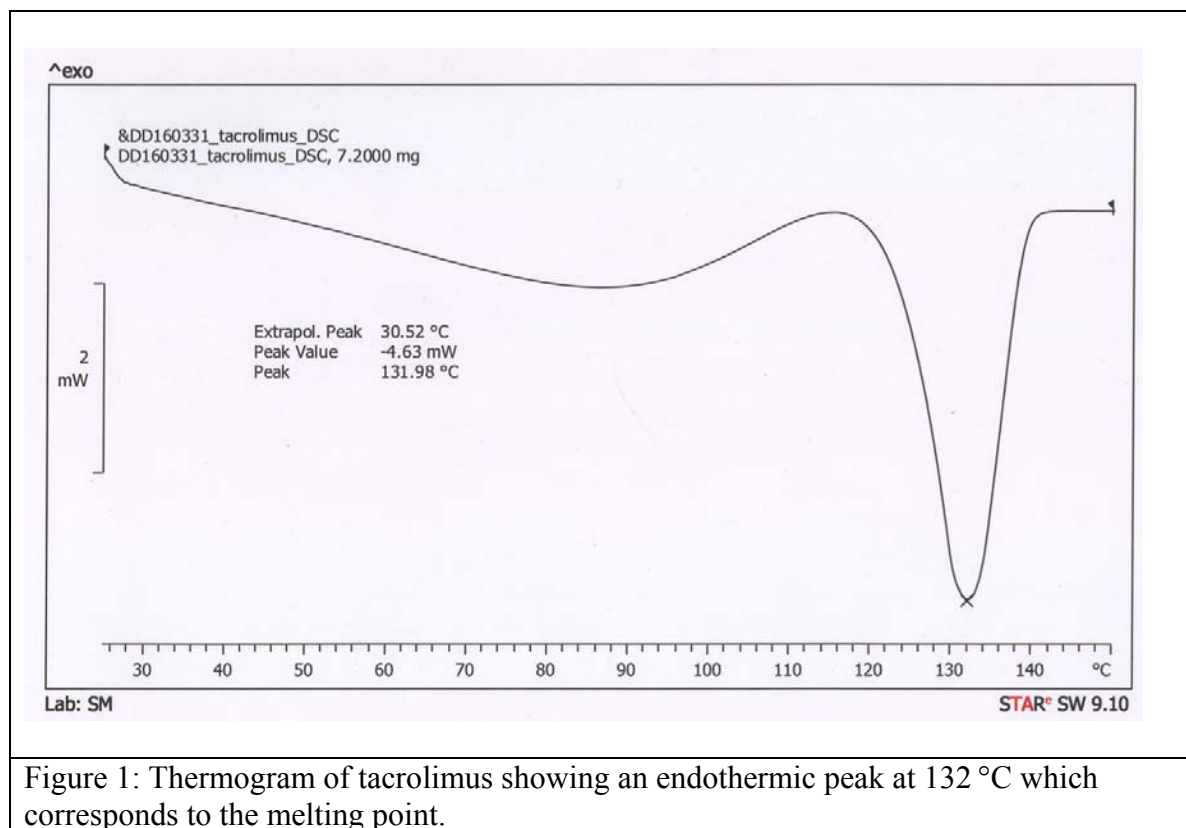
Task 2: To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free upper-extremity facial allotransplantation in a non-human primate (NHP) model.	12-24	
2.2 Fabricate and characterize FK506-PLDS for implantation in-vivo	13	
Task 3: To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for upper—extremity facial allotransplantation in a non-human primate model.	24-36	
3.4 Fabricate and characterize FK506-PLDS for implantation in-vivo	25	

3.2 What was accomplished under these goals?

Major activities. As per the SOW, Task 1.1 is to formulate a polymeric local delivery system (PLDS) for tacrolimus and characterize *in vitro*. Within this task, three subtasks were either completed or work has been initiated and is ongoing, as further described below.

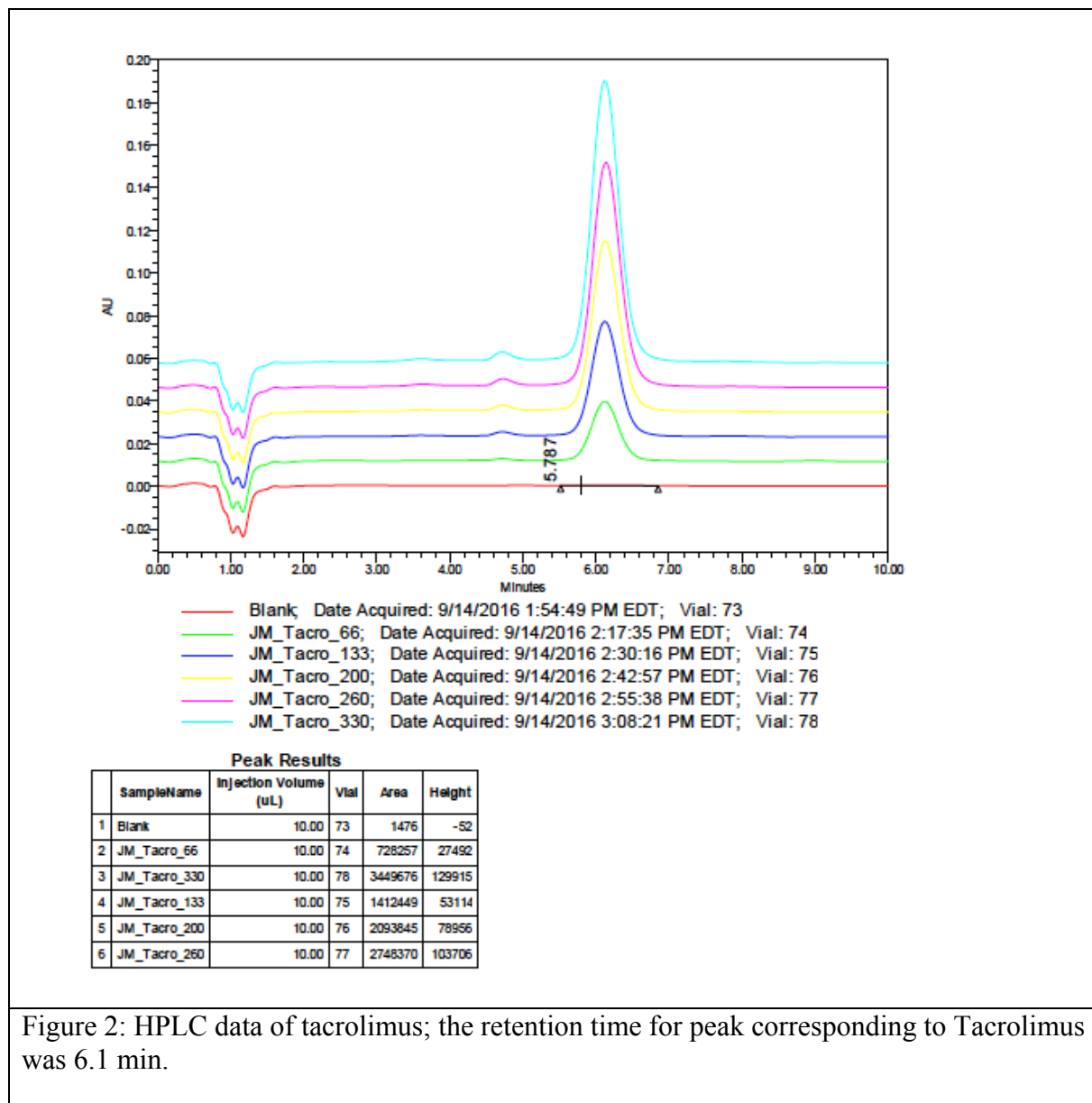
Polymer synthesis and characterization: The polymer formulation, E1218(1k), was chosen due to its proven history. E1218(1k) was previously tested both *in vitro* and *in vivo* as a drug delivery system using voclosporin (VCA), which resulted in several publications including a comprehensive patent that delineated the innovative system design approach for a drug delivery

implant. For this work, E1218(1k) was synthesized and characterized. The chemical structure was confirmed via NMR, and the molecular weight and polydispersity were measured via GPC. *Investigate thermal stability of tacrolimus in the presence of polymer:* As a preliminary study, differential scanning calorimetry (DSC) was used to obtain the melting point of tacrolimus alone. This information was obtained for two reasons, (1) to validate the method by comparing to values found in literature, and (2) to assess the effects of heat on the drug, since compression molding was going to be used (DSC uses heat). A DSC system (2920, TA Instruments, New Castle, DE) with a heating rate of 10°C min⁻¹. Tacrolimus showed an endothermic peak at 132 °C which corresponded to its melting point (Figure 1).



A second analytical technique, high performance liquid chromatography (HPLC), was employed for confirming the presence of tacrolimus. An HPLC system (Waters 2969 Separation Module) with UV/Visible detector (Waters 2489) and C-18 column at 50 °C (Waters SymmetryShield RP18, 3.5 µm, 2.1 x 150 mm) was used in this study. The mobile phase used for elution was 45% water (containing 0.1 % trifluoroacetic acid) and 55% acetonitrile (containing 0.1 % trifluoroacetic acid). The pump flow rate was 0.5 ml/min. As shown in Figure 2, the retention

time for the peak corresponding to tacrolimus was 6.1 min. A calibration curve for tacrolimus was also obtained ($R^2 = 0.9998$), data not shown.



Fabricate tacrolimus-loaded polymeric local delivery system (tacrolimus-PLDS) devices:

The initial work for optimizing the PLDS fabrication method required selecting the appropriate solvent. Although several solvents dissolve either tacrolimus or the polymer, dichloromethane (DCM) is one of the few that dissolves both. Dichloromethane was therefore selected for PLDS fabrication.

The next step was to optimize the PLDS fabrication process in order to reach the targeted thickness of 0.5 mm. Solvent casting, a known method for producing films was chosen. However, the optimal polymer to solvent ratio was not known. In order to identify the optimal ratio, a series of polymer (E1218(1k)) to solvent (DCM) solutions with varying polymer to solvent ratios were prepared, cast into Teflon dishes, dried, and the thickness was measured. Although a thickness close to 0.5 mm was obtained, the fabrication process was difficult due to the high viscosity of the polymer/ solvent combination. The fabrication process was revised to include a less viscous solution for casting, followed by drying and then compression molding.

Films using the optimized fabrication method were characterized using DSC. Table 1 (below) shows the values measured for two films at various locations. Results show low variability in the Tg at the different locations of the sample.

Table 1: Glass transition temperature (Tg) of PLDS.

PLDS Sample Formulation	Film 1 or 2	Region of Sample Analyzed	Tg (°C)
E1218(1k) + Tacrolimus (85:15 ratio)	1	Middle	19.5
	1	Edge	20.9
	1	Edge	22.8
	2	Middle	21.8
	2	Edge	22.9
	2	Edge	22.6
			Average

Measuring the Tg was also necessary for selecting the appropriate heat settings during compression molding. The general steps for compression molding are listed below:

- Carver press was set to 70 °C in order to be approximately 50 °C above the Tg of the drug loaded polymer film.
- Once a steady temperature was achieved, a sample of 10% solids E1218(1k) film was placed on kapton within the mold.
- Shims of 550 µm were placed within the mold.
- Another sheet of kapton was placed on top of the shims and finally the top of the mold was replaced.
- The mold was then placed in the carver press for 5 minutes at atmospheric pressure.
- Pressure was then applied gradually up to 10,000 psi and held for 1 minute.
- Pressure was then released in a controlled manner.

The PLDS fabrication process of solvent casting plus compression molding produced the targeted film thickness of 0.5 mm as well as produced miscible films. This fabrication method has therefore been selected for PLDS batch fabrication encompassing all upcoming work (*in vitro* and *in vivo*). Figure 3 (below) shows photographs during and after the fabrication procedure.

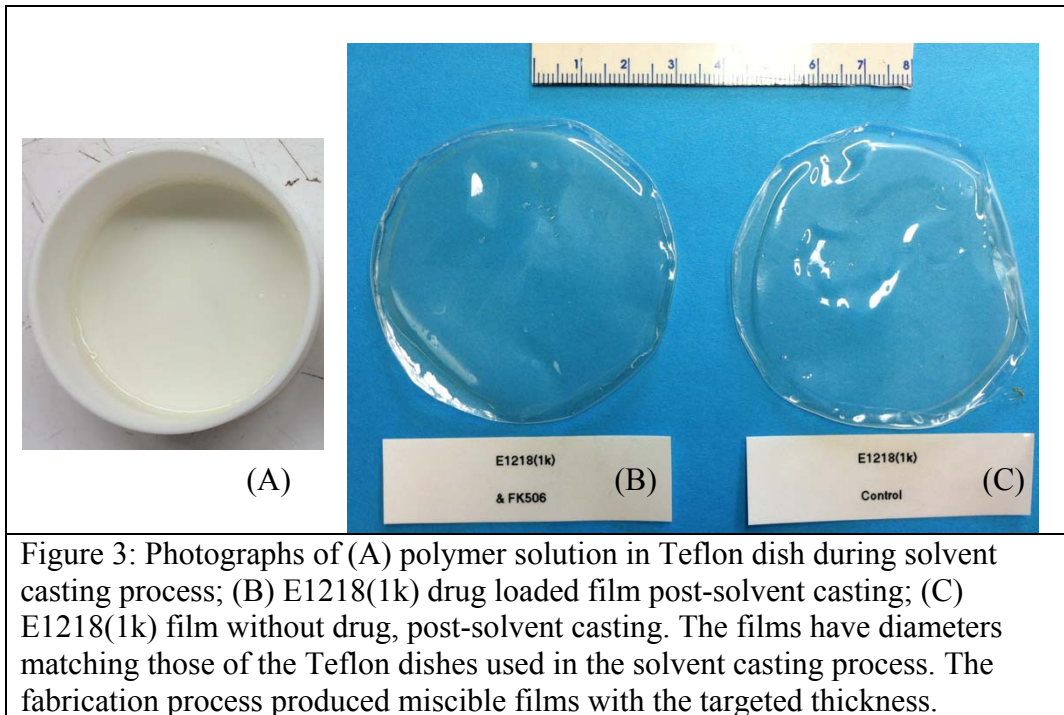


Figure 3: Photographs of (A) polymer solution in Teflon dish during solvent casting process; (B) E1218(1k) drug loaded film post-solvent casting; (C) E1218(1k) film without drug, post-solvent casting. The films have diameters matching those of the Teflon dishes used in the solvent casting process. The fabrication process produced miscible films with the targeted thickness.

Specific objectives

Significant results/ key outcomes

- a) E1218(1k) was synthesized and characterized.
- b) Preliminary devices were made and sent to collaborator for feedback on size, shape, thickness, and handling.
 - a. Based on the feedback, solvent casting plus compression molding has been determined as the optimal method for PLDS fabrication.
 - b. Devices are being fabricated to meet size specifications determined by collaborator.
- c) Analytical method development for determining *in vitro* pharmacokinetics/ drug release has been initiated and is currently underway.
 - a. New HPLC columns were purchased for these studies and have been installed.
 - b. A method has been developed for tacrolimus identification.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

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

The specific objectives for Year 1, to develop and characterize Polymeric Local Delivery Systems for Tacrolimus (FK506) and profile the *in vivo* pharmacokinetics of tacrolimus in a small animal model, were not completed. Due to contractual delays (6 months) and changes in personnel, the project was significantly delayed. The Rutgers Team plans to work diligently to overcome the delays caused by contractual issues and personnel changes. To this end, the Rutgers Team requests the addition of 6 months to the SOW timeline. The Team plans on continuing the work started during Year 1, and by December 2016 will ship samples to the MGH Team for *in vivo* testing in the primate model.

In order to accomplish the tasks at an accelerated pace, both the Rutgers and MGH Teams will have periodic telecons/ communication in order to assess the status of the work and discuss challenges while working through the tasks and deliverables.

4. IMPACT:

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Actual or anticipated problems or delays and actions or plans to resolve them.

The contractual issues during Year 1 caused a delay of 6 months. In addition to this, there were changes in the Rutgers personnel. The team is working to overcome past delays, and requests the addition of 6 months to the SOW timeline.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals.

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

- 6. PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state “Nothing to Report.”

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers, and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Please see Table 2 below:

Table 2: Personnel efforts and person month worked.

Name	Project Role	Person month worked	Contribution to the project
Joachim Kohn	PI	0.60	Administrative and technical oversight of Rutgers team
Sangya Varma	Program Manager	0.60	Oversees project for Rutgers team, edits & completes reports, monitors project progress
Ophir Ortiz	Program Manager	0.10	Oversees project for Rutgers team, edits & completes reports, monitors project progress
Carmine Iovine	Scientist	1.90	Worked on Polymer design, synthesis & experimental design
Ritu Goyal	Post-doc	0.90	Worked on drug encapsulation & characterization of the release profile
Dania Davie	Graduate Student	6.00	Worked on polymer synthesis, drug encapsulation & characterization of the release profile
John Watkins	Technician	0.50	Worked on instrument and computer support
Carol Lenardson	Business Manager	0.50	Managed the award's finances and monitors expenses
Carmen Castro	Senior Coordinator	0.20	Interacts with IACUC/ACURO and compiles reports

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

Nothing to report.

What other organizations were involved as partners?

Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

QUAD CHARTS: Updated and submitted as attachment.



W81-XWH-15-1-0281 Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol

PI: Dr. Joachim Kohn

Org: Rutgers, The State University of New Jersey

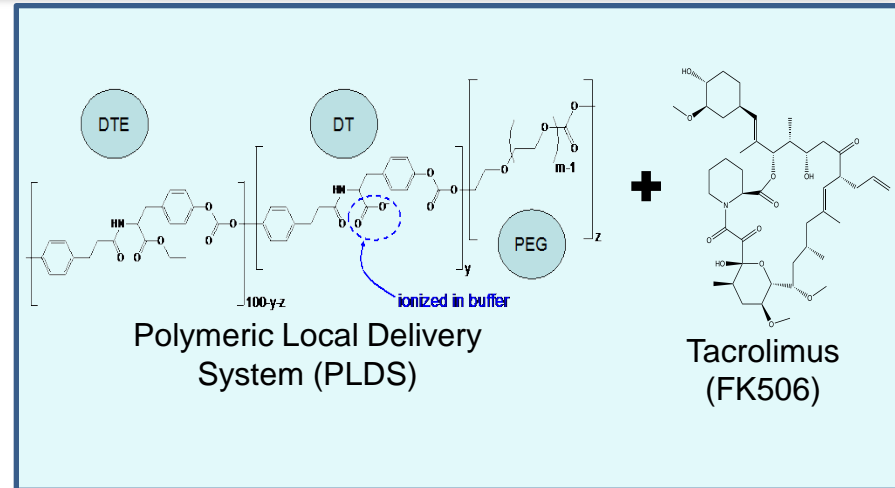
Award Amount: \$300,000.00

Study/Product Aim(s)

Specific Aim 1: To develop and characterize Polymeric Local Delivery Systems for tacrolimus (FK506) and profile the *in vivo* pharmacokinetics of tacrolimus in a small animal model.

Specific Aim 2: To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free facial allotransplantation in a non-human primate (NHP) model.

Specific Aim 3: To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for facial allotransplantation in a non-human primate model.



An appropriate composition of the Polymeric Local Delivery System (PLDS) was selected (70% DTE, 12% DT and 18% PEG (1kD)). The polymer was synthesized and characterized. Approval was obtained for animal protocols relating to Task 1.

Timeline and Cost

Activities	CY	15	16	17	18
Develop PLDS and profile <i>in vitro</i>					
Optimize implant and dose <i>in vivo</i>					
Provide immunosuppression with implant					
Estimated Budget (\$K)		\$56	\$160	\$66	\$18

Goals/Milestones

CY15 Goal – System development and initial *in vivo* testing

- Polymer synthesis and characterization
- Fabricate tacrolimus-loaded polymeric local delivery system
- Obtain IACUC and ACURO approval of animal subcutaneous protocol

CY16 Goals – Optimize the tacrolimus implant + systemic immunosuppression dose regimen

- Optimize the PLDS and dose regimen in non-human primate model

CY17 Goal – Provide adjunctive local immunosuppression with PLDS in a non-human model

- Validate design architecture for digital ear canal volumetric shape

Comments/Challenges/Issues/Concerns

- Due to a delay in sub-award execution, progress has been delayed.
- Addition of 6 months to the timeline is being requested.

Budget Expenditure to Date

Projected Expenditure: \$191K

Actual Expenditure: \$88K