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

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14. ABSTRACT
The focus for Year 3 was to study the controlled release of tacrolimus (FK506) from a polymeric local delivery system (PLDS) both *in vitro* and *in vivo* and the stability of the devices in storage. In the *in vivo* studies, the PLDS released a large amount of tacrolimus, a first in the field which often suffers from low levels of release. Laminated PLDS devices were developed to reduce the initial burst of drug *in vivo* while improving the controlled release profile. A subcutaneous rat model was initiated to efficiently characterize the *in vivo* release profile of the reformulated devices, which now show burst-free controlled release over 7 days *in vivo*. The release profile observed *in vivo* correlates to prior *in vitro* results which is beneficial for screening devices in the future. Long-term storage of the devices leads to a reduction in the release rate of the drug through loss of the molecular dispersion within the film. This helps control the early release rate and provide a more consistent release over time.

15. SUBJECT TERMS
Drug delivery, immunosuppression, tacrolimus, FK506, vascularized composite allografts, immune rejection, preclinical, transplant, nonhuman primate model, degradable polymer, tyrosine-derived polycarbonate

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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 allow 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.*

Table 1. Statement of Work for MGH

Task	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	**1-36**	
1.1. Formulate polymeric local delivery systems (PLDS) for tacrolimus and characterize in vitro.	**1-36**	
1.1.1. Polymer synthesis and characterizations	1	100% complete
1.1.2. Investigate thermal stability of tacrolimus in the presence of polymer	8-15	100% complete
1.1.3. Fabricate tacrolimus-loaded polymeric local delivery system (tacrolimus-PLDS) devices	2-27	100% complete
1.1.4. Investigate the irradiation stability of tacrolimus-PLDS	18-36	100% complete
1.1.5. Perform in vitro polymer degradation and release study of tacrolimus	12-36	100% complete
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.	**1-36**	
1.2.1. Obtain IACUC and ACURO approval of animal subcutaneous implantation protocol	1-3	100% complete
1.2.2. Cohort 1 - Demonstrate in vivo safety of tacrolimus (15wt%) loaded PLDS for 2 weeks and 3 weeks in a rat subcutaneous model. (Fabrication of devices + 3 weeks animals in life + analyses).	5-16	100% complete
1.2.3. Cohort 2 - Optimize methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with systemic tacrolimus.	16-21	100% complete
1.2.4. Cohort 3 - Optimize and finalize test methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with drug-loaded PLDS.	19-36	100% complete
Task 2. To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free partial facial allotransplantation in a nonhuman primate (NHP) model.	**12-27**	
2.1. Obtain IACUC and ACURO approvals.	1-6	100% complete

2.2. Fabricate and characterize FK506-PLDS for implantation in vivo	12-27	100% complete
2.3. Partial heterotopic face transplants on SIS protocol (n=6).	12-24	100%
2.4. Investigate VCA survival, frequency of rejection and complications, document rejection process clinically and histologically.	12-54	100%
2.5. Summarize optimal immunosuppressive requirements for VCA survival in NHPs. Analyse and summarize data on VCA rejection. Year 1 report.	12-24	100%
Task 3. To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for partial facial allotransplantation in a non-human primate model.	**24-54**	
3.1. Delayed tolerance induction protocol, wean immunosuppression.	10-54	75%
3.2. Partial heterotopic face transplants on 2 months SIS (n=4).	24-36	75%
3.3. Investigate chimerism, in vitro immune status, VCA survival outcomes following weaning of immunosuppression.	24-36	100%
3.4. Fabricate and characterize FK506-PLDS for implantation in vivo	36	100%
3.5. Summarize preliminary data/progress on DTIP transplants for inclusion in annual report		100%

Table 1. Statement of Work for Rutgers University (Study Site 2 (sub))

Task	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	**1-36**	
1.1. Formulate polymeric local delivery systems (PLDS) for tacrolimus and characterize *in vitro*.	**1-36**	
1.1.1. Polymer synthesis and characterizations	1	100% complete
1.1.2. Investigate thermal stability of tacrolimus in the presence of polymer	8-15	100% complete
1.1.3. Fabricate tacrolimus-loaded polymeric local delivery system (tacrolimus-PLDS) devices	2-27	100% Complete
1.1.4. Investigate the irradiation stability of tacrolimus-PLDS	18-36	100% Complete
1.1.5. Perform *in vitro* polymer degradation and release study of tacrolimus	12-36	100%
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.	**1-36**	
1.2.1. Obtain IACUC and ACURO approval of animal subcutaneous implantation protocol	1-3	100% complete
1.2.2. Cohort 1 - Demonstrate *in vivo* safety of tacrolimus (15wt%) loaded PLDS for 2 weeks and 3 weeks in a rat subcutaneous model. (Fabrication of devices + 3 weeks animals in life + analyses).	5-16	100% complete

1.2.3. Cohort 2 - Optimize methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with systemic tacrolimus.	16-21	100% complete
1.2.4. Cohort 3 - Optimize and finalize test methods for tacrolimus quantification by testing tissue samples of NHP that have been treated with drug-loaded PLDS.	19-36	100%
Task 2. To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free upper extremity facial allotransplantation in a nonhuman primate (NHP) model.	**12-27**	
2.2. Fabricate and characterize FK506-PLDS for implantation *in vivo*	12-27	100%

Task	**Timeline (months)**	**Comments**
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*	36	100%

3.2 What was accomplished under these goals?

RUTGERS University

1. Fabrication. The primary activity of Q1 was the finalization of the fabrication process for the original PLDS composition. Devices were produced for *in vitro* studies and a subcutaneous rat study. Figure 1 shows photographs of PLDS after the optimized fabrication process.

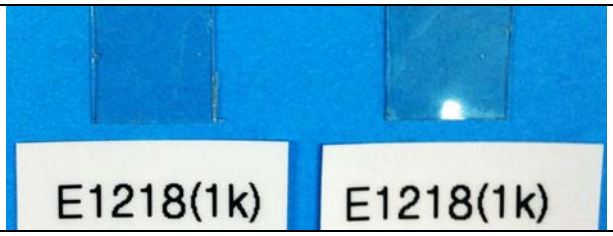


Figure 1. Photographs of PLDS films: Control, without tacrolimus (left) and with 15% tacrolimus (right). Films measure 10 mm (L) x 10 mm (H) x 0.5 mm (W).

2. Thermal Stability. A heat stability study was performed as per Task 1.1.2; the PLDS devices were stable above the temperature required for the fabrication (Fig. 2). Therefore, the team was confident that the fabrication process had adequate parameters for compression molding. DSC scans showed a T_g of 20˚C, compared to 15˚C for the polymer alone, and no drug recrystallization in solvent cast films. Full recovery of tacrolimus from devices treated at 70˚C for up to 480 min was achieved.

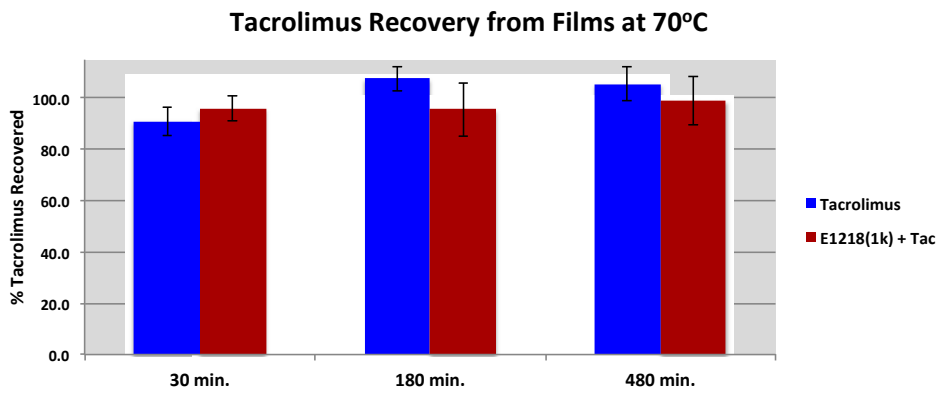
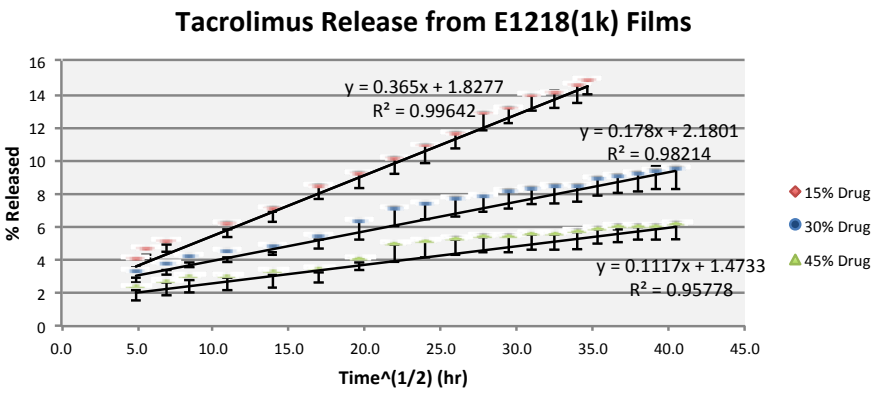


Figure 2. Tacrolimus (FK 506) recovery after heat treatment at 70 ºC for up to 480 minutes. Bulk tacrolimus (blue) was compared to tacrolimus recovered from films (red). As per these measurements, the heat stability of tacrolimus was confirmed to be adequate for the fabrication process of the PLDS films.

3. *In Vitro* Drug Release from the PLDS. A release study measuring the release of tacrolimus (FK506) from 15, 30, and 45 wt% drug-loaded films was completed (Fig. 3). Tacrolimus released into PBS at 37 °C was assessed by analytical HPLC at regular timepoints. At the end of each experiment, the films were dissolved and the remaining tacrolimus content was quantified by HPLC.

Increased drug loading did not lead to a statistical increase in total drug released. By 50 days, all three formulations released 1610-1670 µg. The relationship between loading and release can be attributed to the hydrophobic tacrolimus (FK506) substituting the polymer, which is rich in hydrophilic poly(ethylene glycol) (PEG). The hydrophobicity reduces the hydration of the films and thus the diffusion of the drug out of the matrix. This was confirmed when the percent of drug released was plotted against the square root of time, a linear profile in accordance with Fick's second law of diffusion was obtained, with the slope corresponding to the diffusivity in the matrix.



Fickian Diffusion from a Thin Polymer Film: $\frac{M_t}{M_o} = 4\sqrt{\frac{D_{eff}t}{\pi L^2}}$

Comparison	15:30	15:45	30:45
Drug Ratio	0.50	0.33	0.67
Slope Ratio	0.49	0.31	0.63

D_{eff} (m^2/s): 15% = $2.4x10^{-3}$; 30% = $0.6x10^{-3}$; 45% = $0.2x10^{-3}$

Figure 3. A release study was performed using 15, 30, and 45 wt% tacrolimus (FK506) PLDS. It was confirmed that drug was released over time, in accordance to Fick's Law.

4. Histological Analysis. PLDS were implanted subcutaneously in a rat model for up to 3 weeks (Figure 4), as per Task 1.2. H&E staining of samples confirmed the following:

- Compared to controls, tacrolimus (FK506) treated rats had thinner capsules with less inflammation.
- Compared to controls, tacrolimus (FK506) treated rats had less inflammation separating fascial planes and muscle fibers.

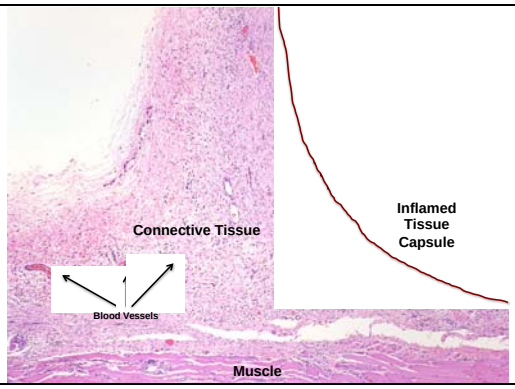


Figure 4a. H&E stained tissue of tacrolimus-loaded PLDS showed minimal inflammatory response relative to the non-drug loaded film.

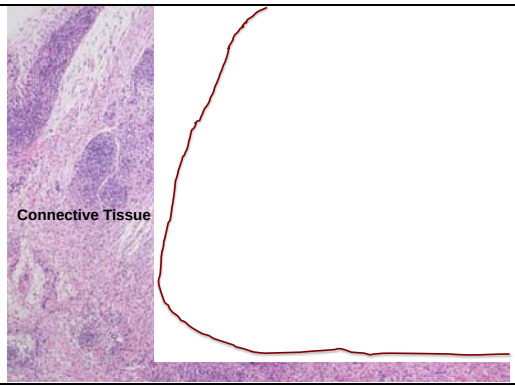


Figure 4b. H&E stained tissue of polymer film without tacrolimus showed an inflammatory response; a thicker inflammatory capsule was observed surrounding the implanted film.

5. *In Vitro* Drug Release from the PLDS. *In vitro* release studies on 15% tacrolimus-loaded E1218(1k) films in PBS at 37˚C were followed out to 16 weeks. On average, a total of 2.17 +/- 0.05 mg of tacrolimus was released from a 1 cm^2 film over the course of the study, which corresponds to 23% of the loaded drug (Fig. 5). Drug release occurred in three stages: an initial burst stage, an early-stage release and a late-stage release. Over the first day, a burst of 0.67 mg (7.2%) of drug was released. By the end of the first week, the burst release has dissipated and a total of 1 mg (11%) of the drug was delivered. Over weeks 2 to 6, drug was released at a consistent rate, bringing the net release to 1.88 mg (20.3%). At six weeks, release transitioned into a late stage profile marked by a further reduction in the release rate, with 0.29 mg released over weeks 7-16. By week 16, the cumulative release of tacrolimus was 2.17 mg (23.6%) from a 1 cm^2 film.

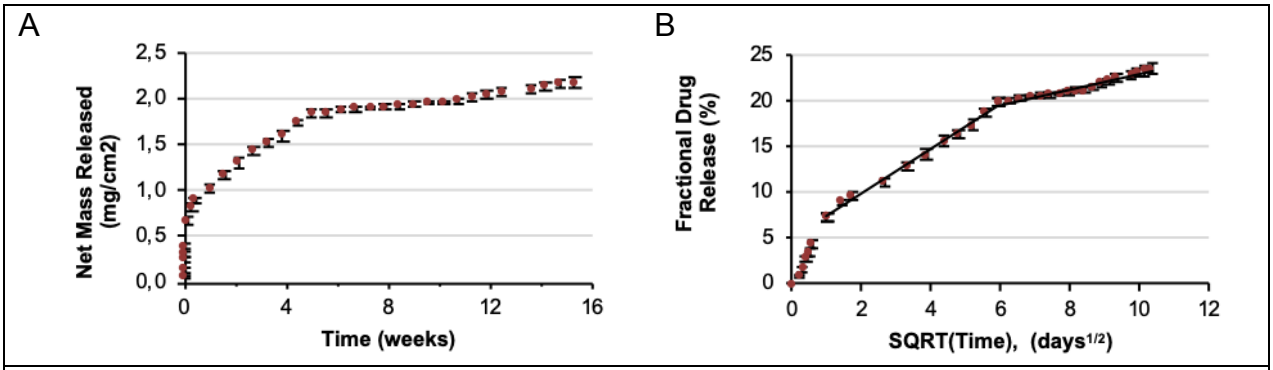


Figure 5. (A) Release profile of tacrolimus from 15% tacrolimus (FK506) loaded film over 16 weeks. (B) Fractional release profile of 15% tacrolimus (FK506) loaded films plotted against the square root of time to show the three stages of release.

6. *In Vivo* Drug Release - Quantification. A protocol for analyzing tacrolimus concentration extracted from animal tissue was developed in collaboration with the Rutgers University Biological Mass Spectrometry Facility. LC-MS/MS was used to measure drug concentration by comparison to pre-defined standard curves. The limit of detection for this method was between 0.1 and 0.05 ng/ml. Initial tissue samples from a primate receiving systemic tacrolimus dosing showed large variations in tacrolimus levels on a mass/mass basis (Fig. 6). This deviation was attributed to the biopsy location, tissue physiology, and hydration.

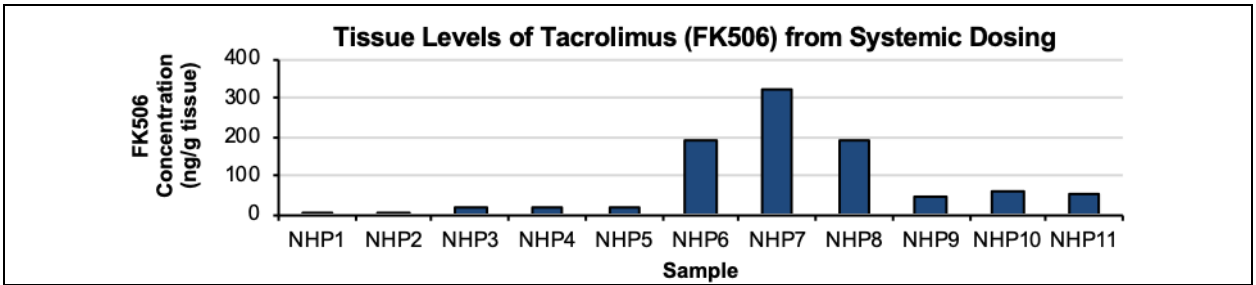


Figure 6. Tacrolimus concentration in tissue explants on a mass of tacrolimus (FK506) to mass of tissue basis (ng/g). All samples received were from one primate.

7. *In Vivo* Drug Release – Small Animal Model. The diffusion profile of tacrolimus *in vivo* was investigated to inform the placement of the films in the NHP model. Three films were

implanted subcutaneously in rats for 7 days. After 7 days, the animals were sacrificed and the tissue surrounding the implants was surgically removed. Six 3 mm biopsies were collected at 5 and 10 mm from the implant, using a systematic collection grid (Fig. 7). Samples were subject to the standardized homogenization-extraction protocol and analyzed by LC-MS/MS. The results were inconclusive as the diffusion in the rat model also exhibited high variability, as observed in the NHP model (Figure 6). This was potentially due to underlying tissue physiology, including the presence of blood vessels and/or fatty tissue (Fig. 7). We can conclude that the drug is released from the films *in vivo* and is present at least 10 mm away from the implant site.

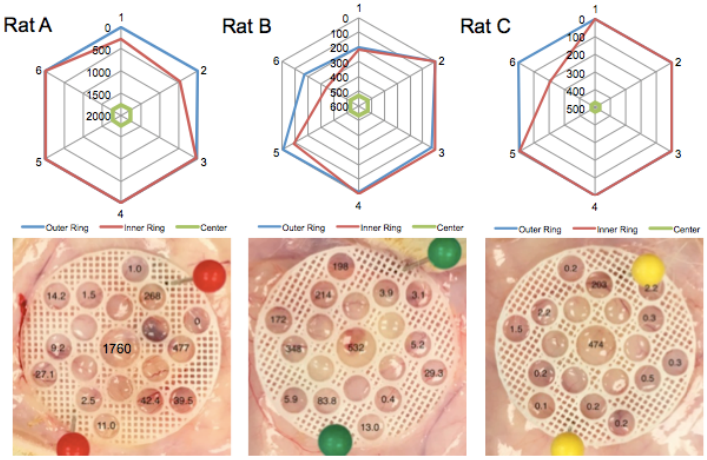


Figure 7. (a) Radar plots showing drug concentration of the areas surrounding the implanted PLDS after 7 days. Drug concentration drops off rapidly and is not distributed evenly around the devices likely due to underlying tissue characteristics, such as the presence of blood vessels and fat. (b) Overlay of the measured drug concentration at each site where the corresponding tissue biopsy was removed.

8. *In Vivo* Drug Release – NHP Model 1. The characterized 15% tacrolimus-containing E1218(1k) devices were implanted in a non-human primate VCA surgery performed at Massachusetts General Hospital. The number of implanted devices was determined based of the release profile, tissue distribution and standard intravenous dosing protocol where the amount given to each animal is 0.1 mg/kg/day. This was extended over 90 days to give:

$$\textbf{\textit{Intravenous}}: \quad 0.1\,\frac{mg\ Tac}{kg * day} \times 10\,\frac{kg}{primate} \times 90\ days = \mathbf{9}\,\frac{\boldsymbol{mg\ Tac}}{\boldsymbol{primate}}$$

To provide the same overall loading, we calculated:

$$\textbf{\textit{PLDS Implants}}: \quad 0.15\,\frac{mg\ Tac}{mg\ film} \times 60\,\frac{mg\ film}{device} \times 10\,\frac{devices}{primate} = \mathbf{9}\,\frac{\boldsymbol{mg\ Tac}}{\boldsymbol{primate}}$$

Ten 15% drug-loaded devices were implanted at least 20 mm apart around the transplant site. With ten implants, each animal received the same drug loading as is normally administered intravenously over the course of 90 days. However, using our PLDS approach, the release would be extended to later time points and localized to the site of implantation.

The first set of surgeries was completed in April 2017 (Fig. 8). Each PLDS device was sterilized with Betadine immediately before implantation at the transplant site. The primates' appearance and blood tacrolimus concentration levels were monitored for two weeks following the surgeries. One graft failed by POD 4 due to necrosis of the tissue unrelated to the

implanted films (venous congestion due to vein thrombosis). The tacrolimus concentration in the blood was measured up to 12 days post-operation in the second animal and ranged from 50-120 ng/ml (Fig. 8). This value exceeded the recommended therapeutic range of 20-30 ng/ml by 2-6 fold.

9. PLDS Reformulation: Eliminating Burst Release. The *in vivo* tacrolimus release profile in the non-human primate M1417 was not predicted by our *in vitro* data. The high initial levels of tacrolimus that dropped by day 6 and increased levels thereafter indicated that our devices require significant further characterization and redesign. In response to the initial *in vivo* work, our next goal was to reduce the early stage release of tacrolimus to reduce the immunosuppressive burden on the animal. Three solutions were investigated: (1) pre-soaking the devices to wash away free and loosely bound tacrolimus, thereby reducing the magnitude of the initial burst release, (2) reducing the drug loading of each device to reduce the overall drug burden on the system or (3) laminating each film with a thin layer of drug-free polymer on both the top and bottom to seal in the drug and provide a diffusional barrier to prevent the initial burst release.

Prewashing: 15% films were prewashed in DI water for 24 hours and dried overnight.
Reduced Loading: 5% and 10% drug-loaded devices were fabricated using the standard protocol.
Lamination: Drug-free solutions of 70 mg/ml E1218(1k) in DCM were solvent cast into films and dried using the same methods as used for the drug loaded films. The final thickness of these drug-free laminating films was 70 μm. The laminated device was prepared by placing a standard 15% tacrolimus loaded device between two drug-free films and heated using the compression molding plates with 550 μm shims at 70^{o}C for 5 minutes at no additional pressure.

The release from the modified devices was tested *in vitro* in PBS at 37˚C and quantified by HPLC. The prewashing protocol reduced the burst release of the films by removing drug close to the surface and in open areas of the matrix (Fig 9). Reduced loading effectively limited the net tacrolimus release by cutting the drug available for diffusion out of the polymer matrix. Laminated films gave zero-order release kinetics at early time points where burst release is usually observed, followed by a reduction of release rate after 24 hours. For the most controlled and effective therapy, it would be beneficial to extend the zero-order release to later time points. It was hypothesized that this may be achieved by increasing the drug loading within the laminated films, a path investigated in Year 2 Quarter 4.

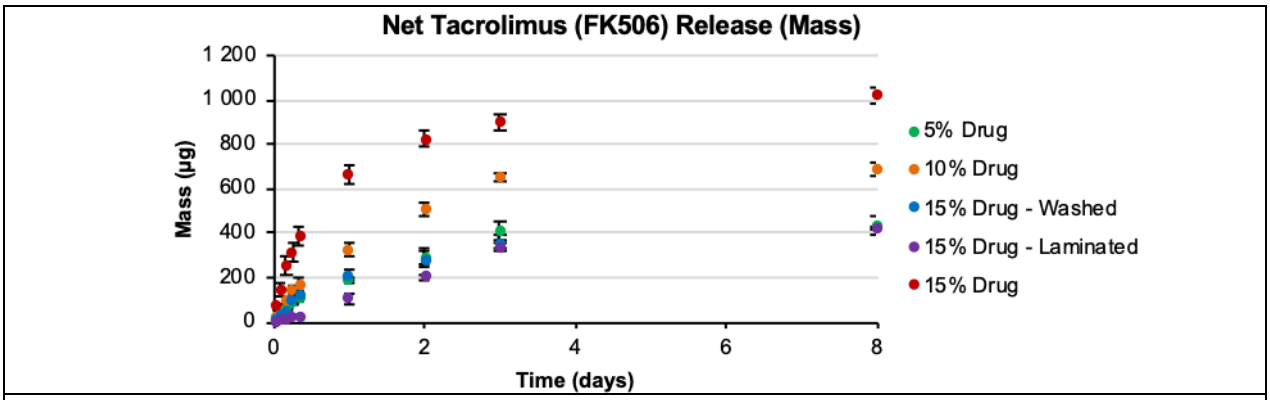


Figure 9. Early *in vitro* release profile of tacrolimus (FK506) from films intended to reduce the initial release rate of tacrolimus into the body. Laminated 15% loaded films showed promising early results.

10. PLDS Reformulation: Lamination. Laminated PLDS devices were selected based on the promising zero order release kinetics at early time points (Fig. 9). Laminated samples underwent lap shear and peel testing (Fig. 10a) to ensure that the lamination process was complete and mechanical forces on the devices would not cause delamination during transportation and handling. Scanning electron microscopy was used to check for an interface between the drug-free and drug containing layers of the device, which would affect the diffusional properties of the drug through the matrix (Fig. 10b). When viewed at high magnification, no interface was observed indicating that the heat molding process created a smooth transition between layers. We concluded that the laminating process was effective at creating stable and reproducible devices.

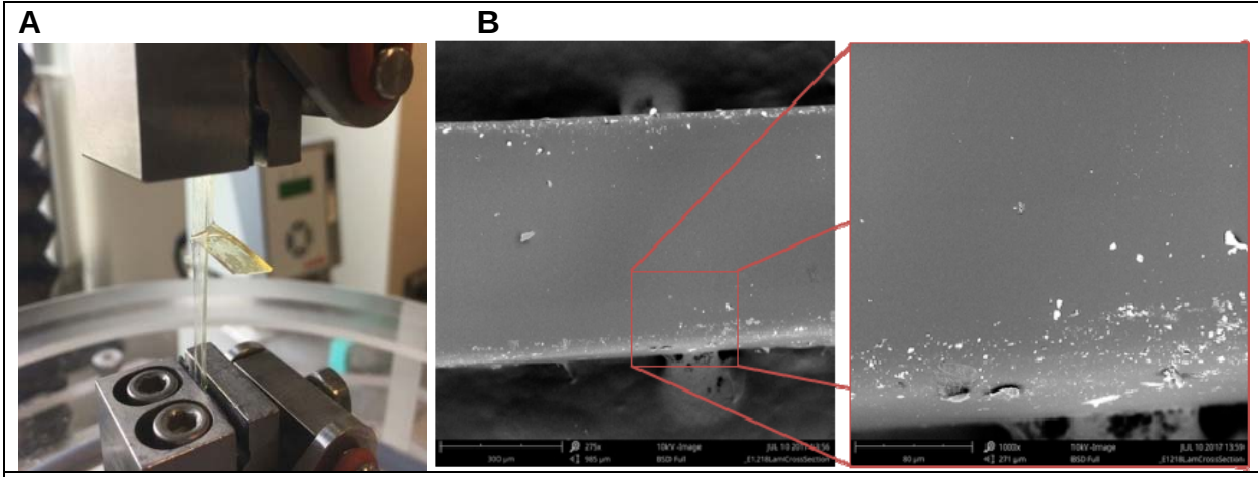


Figure 10. (a) Polymer stretching occurs during peel testing of a laminated device before any delamination occurs, indicating that a strong interlayer bond has formed. (b) SEM imaging of a cross-section of laminated film shows no visible interface between the layers of polymer.

We next studied the long-term release from laminated films with a range of drug loading to determine if increasing the amount of drug would help extend the zero-order release to later time points. Laminated films were produced with 15%, 30% and 45% tacrolimus by weight and three 10 mm diameter disks were cut from each using a 10 mm diameter disposable biopsy punch. These disks were immersed in PBS and incubated at 37˚C. The PBS collected at each

time point was analyzed by HPLC to determine the tacrolimus concentration and release over the previous time frame (Fig. 11). Lamination effectively eliminated the early burst release at all drug-loading levels as shown in Fig. 11. Beyond three days, the laminated films follow a similar release profile to the non-laminated films for the duration of the study. The amount of drug loaded in the device had no significant effect on the net amount of drug released among laminated devices, similar to what was observed with the non-laminated devices in an earlier study.

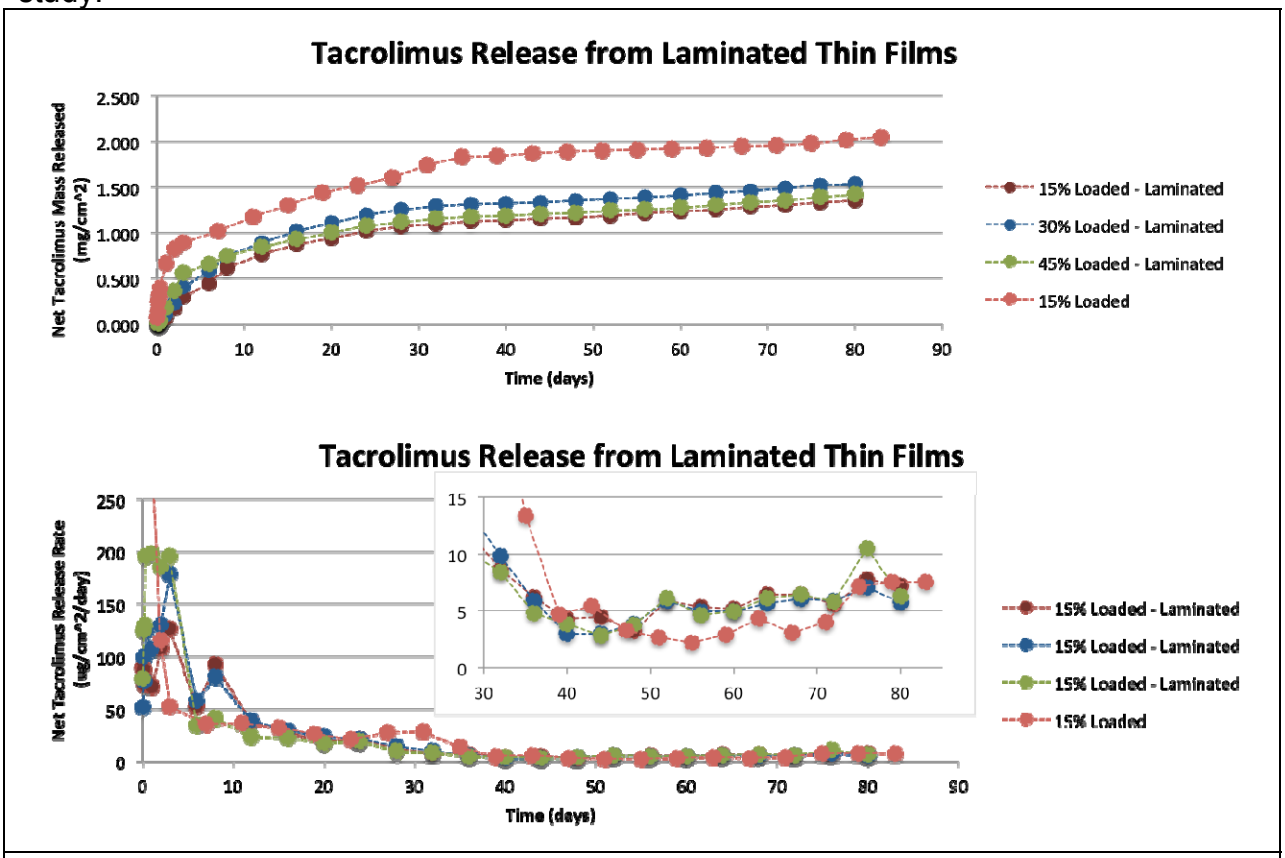


Figure 11. Comparison of net release and release rate of tacrolimus from laminated device with 15%, 35% and 45% tacrolimus loading with 15% non-laminated devices. Laminated devices exhibit a reduced burst release early and a similar profile to non-laminated devices after 72 hours.

11. *In Vivo* Drug Release – NHP Model 2. Laminated 1 cm x 1 cm devices loaded with 15% tacrolimus were supplied to MGH for a follow up study in a NHP VCA model. In order to determine the effect of dosage on systemic levels and associated graft rejection, two devices were used in the first primate, M1517, while one device was used in the second primate, M1617 which is equivalent to a dosing of approximately 0.9 mg/kg M1517 and 1.8 mg/kg for M1617. This approximately models the daily intravenous dose with the goal of also extending the localized immunosuppression for as long as possible. Reducing the burst release through lamination and the number of implants brought the systemic concentrations for the second round of NHP surgeries within desired therapeutic range (Fig 12). Results were particularly promising for NHP M1617 where systemic tacrolimus concentration due to the implanted films were maintained near the 20-30 ng/ml range out to day 11, at which time additional tacrolimus was administered intravenously to bolster the immunosuppressant levels. M1517 was sacrificed on POD 5 due to venous congestion of the VCA (vein thrombosis). Extending what

is effectively a two day intravenous dose out to 11 days while maintaining a therapeutic dose represents a 5x reduction in the amount of tacrolimus required for treatment over that time period. This is highly impactful in that it could significantly reduce the prevalence of systemic side effects while eliminating the need for repeated injections of tacrolimus. Furthermore, the localized concentration of tacrolimus within the VCA is higher than systemic levels, extending effective localized treatment beyond the 11 days indicated by systemic blood concentration.

12. PLDS Reformulation: Gamma Sterilization and Stability.

Drug loaded films, pure tacrolimus and pure E1218(1k) polymer were sterilized by gamma irradiation at Sterigenics. Samples were exposed to a 33 kGy dose of radiation. Inspection of the devices, polymer and drug showed no visual changes in color, texture, or clarity. Gamma-irradiated 15% drug loaded films are currently being tested for their release characteristics *in vitro* at 37˚C. Any changes in the HPLC curve profile, elution time, and net release over time would indicate radiation-induced chemical changes between the polymer and drug. To avoid gamma-irradiation induced damage to the tacrolimus or polymer, PLDS systems were produced with the addition of % vitamin E, a known free radical stabilizer. The addition of vitamin E had no effect on the release profiles (Fig. 13).

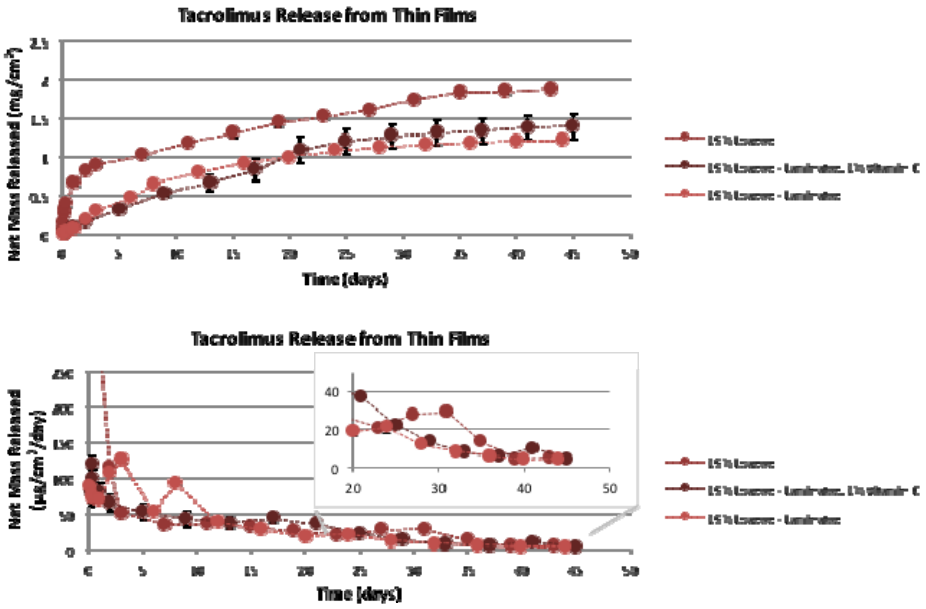


Figure 13. Release of tacrolimus from laminated films containing vitamin E as a free radical stabilizer matched release from laminated films without vitamin E indicating vitamin E can be used in the formulation to help prevent degradation during gamma sterilization without changing the release profile.

13. *In Vivo* Drug Release – Small Animal Model.

In order to better understand the correlation between our *in vitro* studies and *in vivo* results, an *in vivo* study in rats with the laminated devices was developed, monitoring systemic tacrolimus levels following implantation for extended time periods. The goal being to ultimately relate this data back to the *in vitro* results to better predict the number and composition of films required for effective immunosuppression in future NHP studies. Laminated PLDS devices containing 0%, 15%, 30%, and 45% tacrolimus by weight, with and without 1% vitamin E, were gamma

sterilized by Sterigenics, at a lower dose of 22 kGy, sufficient to clinically sterilize similar PLDS devices.

An ongoing subcutaneous study in 16 Sprague-Dawley rats was performed to analyze the *in vivo* release from four different PLDS formulations:

- 0% tacrolimus/1% vitamin E
- 15% tacrolimus/1% vitamin E
- 30% tacrolimus/1% vitamin E
- 30% tacrolimus/0% vitamin E

One 4 mm disk was implanted in a dorsal subcutaneous pocket. Blood was collected daily for the first week and weekly thereafter. Blood samples were sent to MGH for analysis on the same machine used for the NHP studies, an Abbott Architect Immunoassay Analyzer. The weight of all animals containing drug-loaded implants decreased between the day of surgery and POD 7. Animals in the drug-free control group maintained their weight over the same timespan. This indicated that tacrolimus was being released from the devices and having a systemic effect on the animals. Systemic tacrolimus concentrations were 5-15 ng/ml, and did not elicit a toxic burst release (Fig. 14). The release is stable over 7 days, independent of drug loading and the presence of vitamin E.

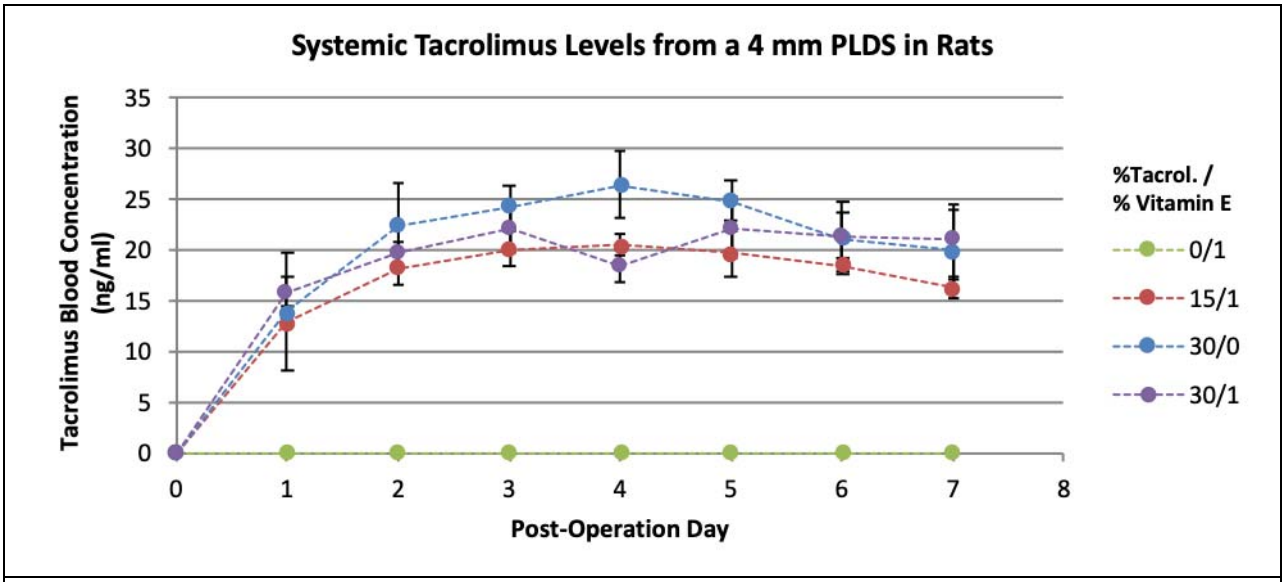


Figure 14. Systemic tacrolimus levels in blood collected from the rats, showing sustained release over 7 days. Rats were implanted with a single 4mm diameter PLDS in a dorsal subcutaneous pocket. This study is ongoing.

MGH

Year 1 and 2 :

The first set of surgeries was completed in April 2017 (Groupe 1 and 2). Each PLDS device was sterilized with Betadine immediately before implantation at the transplant site. The primates' appearance and blood tacrolimus concentration levels were monitored for two weeks following the surgeries. Two graft failed (M1417 and M1517) by POD 4 due to necrosis of the tissue unrelated to the implanted films (venous congestion due to vein thrombosis). The tacrolimus concentration in the blood (**Figure 1**) was measured up to 12 days post-operation in the second animal and ranged from 50-120 ng/ml. This value exceeded the recommended therapeutic range of 20-30 ng/ml by 2-6 fold.

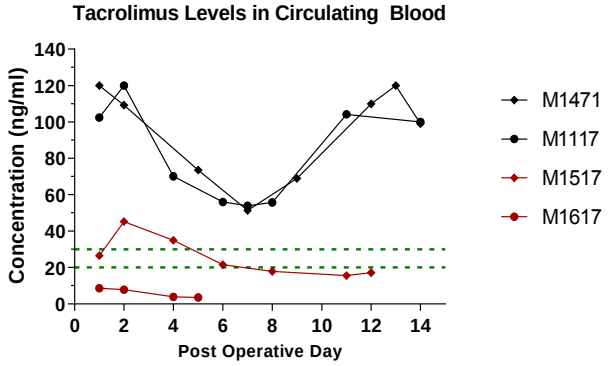


Figure 1: Tacolimus level in circulating blood

The high levels were initially attributed to an IV injection of 1.8 mg of tacrolimus during surgery in addition to the burst release often observed during the first few days *in vitro*. However, the sustained high release past the first week after implantation was cause for serious concern. Clinical assessment of the animal suggested potential side effects due to high tacrolimus levels including poor appetite, weight loss, and a slight tremor. Following explantation, it was observed that the tacrolimus disks were encapsulated in inflamed fibrous tissue. Encapsulation and inflammation were observed in previous *in vivo* studies in rats and may have been exacerbated by the Betadine sterilization method used at the time of implantation.

In group 3, we transplanted (**Figure 2**) (heterotopic partial face transplant) 2 recipients NHP; M8318 and M8418. This report describes the follow-up postoperatively. M8318 was transplanted on 2/19/19 (donor M8218). The surgery was uneventful. Tacrolimus was injected IM daily at the dose of 0.1mg/kg from POD 0 to POD 3. The rest of the immunosuppressive regimen was started as well (ATGAM, MMF, methylprednisolone), and Heparin was administered the first week. The tacrolimus level in the peripheral blood was at 79.1ng/mL on POD 3 when the injections were stopped. On POD 5, an erythema started on the VCA associated with a leukocytosis (11.2G/L), that was treated by administration of Ceftriaxone 50mg/kg IM in the hypothesis of a cutaneous infection (after discussion with the veterinarian staff). Under this antibiotic course, the leucocytosis decreased, and the erythema decreased homogenously but remained at the distal part of the flap. The bacteriological samples came back positive for Enterobacter Cloacae and MRSA. Decision was made to treat by Vancomycin

IV infusion every other day until CBC became normal. After 3 IV infusion (20mg/kg), the flap was looking totally healthy, and the WBC went back to a normal range (6.8G/L). The skin biopsy taken during the erythema episode showed no sign of acute rejection according to the Banff scale. On POD 10, Tacrolimus injection were started again at 0.1mg/kg because of tacrolimus blood levels continuously decreasing from 79.1ng/mL (POD 3) to 26.6ng/mL (POD 10).

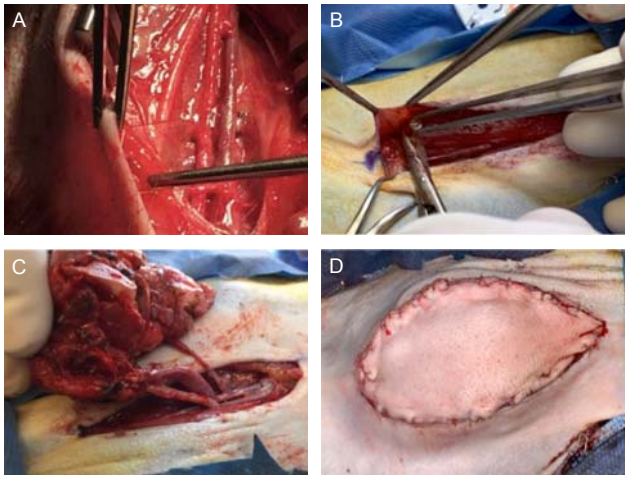
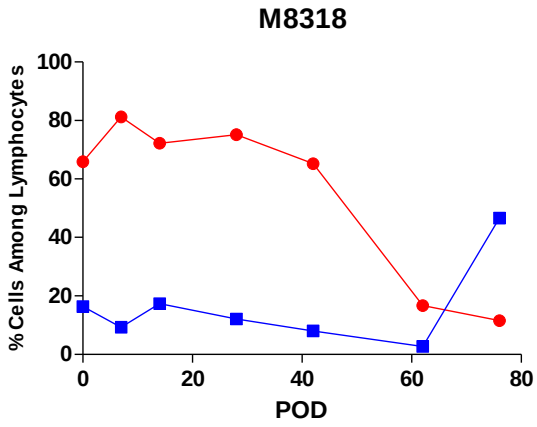


Figure 2: discs implantation in VCA

Of note, he continuously lost weight during the post-operative period despite high protein diet (4.5kg to 4.14kg), then stabilized on POD 29 and went back to 4.46kg on POD 50, helped by oral dronabinol 2.5mg daily. He received total body irradiation (TBI 1.5Gy) on POD 57 and 58, and thymic irradiation on POD 62. Bone marrow transplant on POD 63 was uneventful and $1.023x10^9$ viable cells were infused in the OR under monitoring. After that, the weight dropped again to less than 4 kg. Prophylactic antibiotics (Baytril) were started because of leucopenia. On POD 72, he started gaining weight again. On POD 77, he was sedated for IV infusion of anti-IL6R. After sedation he showed poor recovery, his CBC show 37.4G/L leucocytes and the decision was taken with the vets to euthanize him in front of this suspicion of post-transplant lymphoproliferative disorder (PTLD). The histology confirmed the diagnosis of PTLD and the FACS analysis revealed that it was B-cells (**Figure 3A)** from recipient origin (**Figure 3B**).



T-cells (CD3+)
B-cells (CD20+)

Figure 3A: Whole blood analysis of M8318. Graphs represent % of T and B-cells in the lymphocyte gate at each time point. M8318 B-cell increase between POD60-EOS likely PTLD.

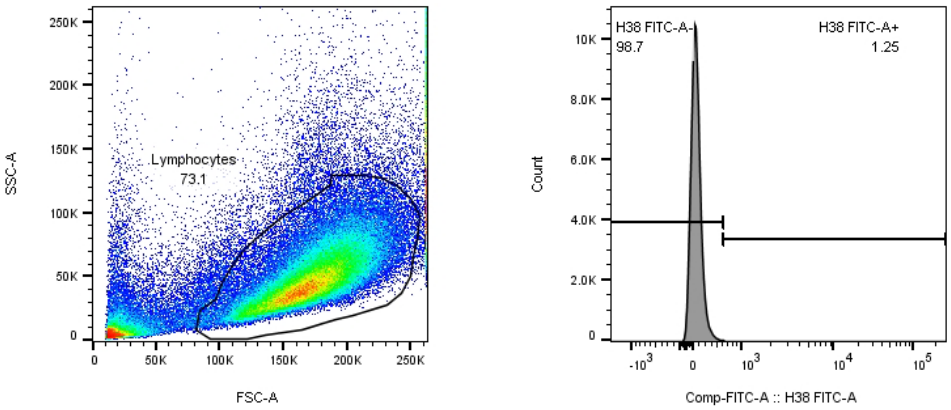


Figure 3B: only H38- cells were detected on the bloodstream demonstrating the recipient origin of the PTLD

M8418 was transplanted on the 2/20/19 (donor M8518). The surgery was uneventful. Tacrolimus was injected IM daily at the dose of 0.1mg/kg from POD 0 to POD 3. The rest of the immunosuppressive regimen was started as well (ATGAM, MMF, methylprednisolone), and Heparin was administered the first week. The tacrolimus level in the peripheral blood was at 112.4ng/mL on POD 3 when the injections were stopped. On POD 4, an erythema was observed on the periphery of the VCA, and then on POD7 the whole flap showed erythema with leukocytosis (19.3G/L) that was treated by Baytril 5mg/kg IM in the hypothesis of a cutaneous infection. On POD 8, a wound dehiscence happened on the distal part of the flap, that was trimmed and sutured with approval from the veterinarians' team. On POD 10 the wound opened again in the same place, we decided to let it heal secondarily. The bacteriological samples came back positive for Staphylococcus sp, decision was made to switch to Trimethoprim 138mg. The erythema disappeared and the wound healed without issue. On POD 14 WBC were normalized. On POD 24, a mild scrotal edema started. Our hypothesis was a side effect of the high tacrolimus level released by the discs. Decision was made to monitor and treat on demand with Furosemide IM. On POD 29, he started looking slower and his WBC increased again to 14G/L, Baytril IM was started again. We discovered a diabetes with glycemia 389mg/dL but no ketones in urines. We started insulin and diabetes diet. After 10 days of antibiotics with still leukocytosis, Ceftriaxone was added. On POD 50, Dronabinol was added to stimulate appetite, and insulin was stopped. During that period of time he significantly lost weight. From POD 50 to POD 60, he started gaining weight. He received total body irradiation (TBI 1.5Gy) on POD 56 and 57, and thymic irradiation on POD

61. Bone marrow transplant on POD 62 was uneventful and $3.21x10^9$ viable cells were infused in the OR under monitoring. On POD 65, tacrolimus IM injection were started again because tacrolimus level was dropping under 20ng/mL. After that, the weight dropped again to less than 4 kg. Prophylactic antibiotics (Baytril) were started because of leucopenia on POD 69. On POD 76, he received a whole blood transfusion to treat his anemia (HBG 6.4g/dL). On POD 72, he started gaining weight again. On POD 83, his weight dropped. On POD 86, sedation planned with the veterinarians for clinical examination and weight assessment. He was still losing weight with a total weight loss of 31% since surgery and NHP looking uncomfortable. Decision was taken to euthanize the monkey. Necropsy showed very unhealthy lungs. The VCA itself contained some lumps. (**Figure 4A and 4B**) Some centimetric lymph node found in inguinal both sides, mesenteric and para aortic. The blood chimerism analysis showed for a high level of chimerism from POD 60 (**Figure 5**).

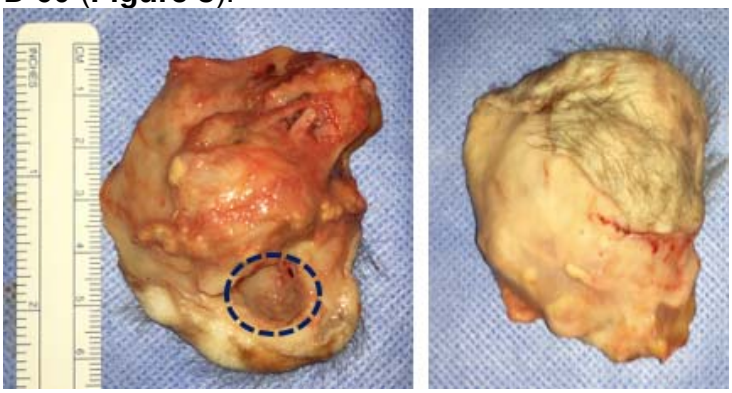


Figure 4A: M8418 developped a posttransplant lymphoma disease (PTLD) inside the VCA.
(left) Posterior view of the VCA (PTLD surrounded by black dashes)
(right) Anterior view of the VCA

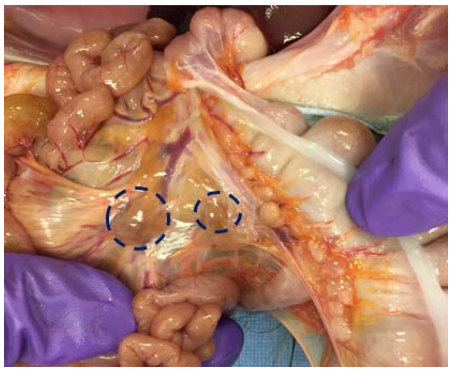


Figure 4B: Intestinal location of PTLD (PTLD surrounded by black dashes)

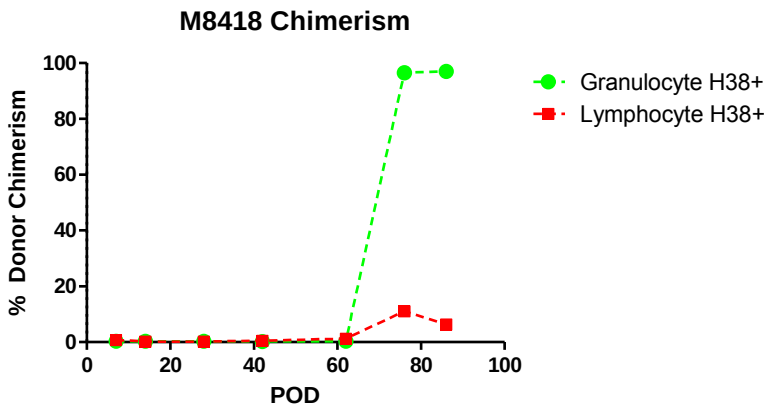


Figure 5: %H38+ donor cells in the granulocyte and lymphocyte gates of M8418 throughout the study. BMT was performed on POD62

In group 3, we transplanted (heterotopic partial face transplant) the last recipient NHP; M5319. This report describes the follow-up postoperatively. M5319 was transplanted on 12/11/19 (donor M5519). The surgery was uneventful. 25 tacrolimus discs were placed subcutaneously. Tacrolimus was injected IM daily at the dose of 0.1mg/kg from POD 0 to POD 2. The rest of the immunosuppressive regimen was started as well (ATGAM, MMF, methylprednisolone), and Heparin was administered the first week. The tacrolimus level (Figure 1) in the peripheral blood was at 55.3ng/mL on POD 2 when the injections were stopped. We gave a prophylactic antibiotic by administration of Ceftriaxone 50mg/kg IM to prevent cutaneous infection (after discussion with the veterinarian staff). No acute rejection was diagnose so far (**Figure 6**).

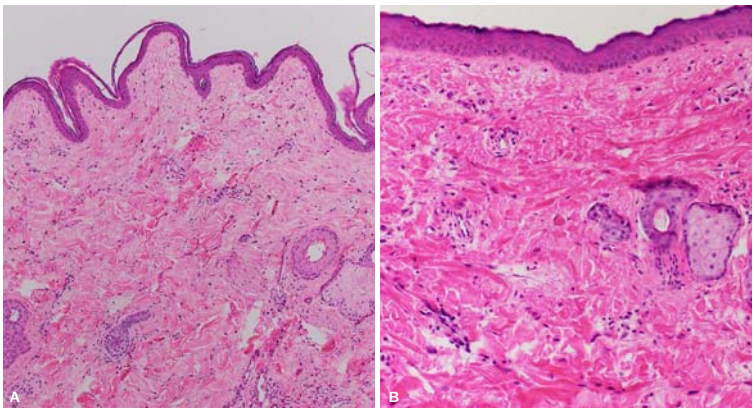


Figure 6: A) 8318 (H&E, 100x) and B) 5319 (H&E, 400x). The skin from the VCA shows no evidence of rejection at the end-of-study. The epidermis is intact and shows no inflammation.

3.3 Specific Objectives

Significant results/ key outcomes

a) PLDS devices were manufactured, sterilized and stored for future use
    a. Stability testing was carried out on stored samples finding that release decreased with storage.
    b. Reduction in release was related to crystallization of the drug within the films and loss of a molecular dispersion.
    c. A veriaty of methods were inacted to prevent this from occurring. The most promising being the incorporation of drug-loaded nanospheres into water soluble films.

b) *In vitro* modelling of the release profile.
    a. The HPLC protocol developed in Year 1 for tacrolimus was used to great effect to quickly and efficiently determine the release profile of the original and modified devices *in vitro* up to 140 days.
    b. The *in vitro* modelling allowed for the characterization of aged PLDS devices which showed reduced release and prolonged the period of controlled release.

c) *In vivo* modelling of the release profile.
    a. A subcutaneous rat model was initiated under existing MR141092.01 (IACUC #94-048) to determine the *in vivo* release profile of the devices.
    b. *In vivo* release was found to correlate to the *in vitro* release profile with a high early release transitioning to a lower late-stage release.

What opportunities for training and professional development has the project provided?
Nothing to report.

How were the results disseminated to communities of interest?
Results were presented as a full-length research talk at the Society for Biomaterials Annual Meeting in Atlanta, Georgia on April 11-14, 2018

Molde, J., Steele, J., Ortiz, O., Iovine, C., Dube, K., Merolli, A. Yang Ng, Z., Certulo Jr., CL., & Kohn, J. Development of Tacrolimus-Loaded Polymeric Delivery System for Localized Immunosuppression. Talk presented at the Society for Biomaterials Annual Meeting; 2018 April 11-14; Atlanta, GA.

Results were presented as a research talk at the 14TH Congress of the international society of vascularized composite allotransplantation ISVCA,October 1st, 2019 at the Main Conference India Exposition Mart, Greater Noida, New Delhi.

Taveau C, Lellouch AG, Andrews AR, Molde J, Ng ZY, Tratnig P, Jonczyk MM, Randolph MA, Kohn J, Cetrulo CL Jr Local FK506 Implant Technology in VCA – Successful Bridge to Delayed Mixed Chimerism 14TH Congress of the international society of vascularized composite allotransplantation ISVCA,October 1st, 2019 at the Main Conference India Exposition Mart, Greater Noida, New Delhi.

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

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.
Storage concerns were addressed and the project is complete.

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.
Molde, J., Steele, J., Ortiz, O., Iovine, C., Dube, K., Merolli, A. Yang Ng, Z., Certulo Jr., CL., & Kohn, J. Development of Tacrolimus-Loaded Polymeric Delivery System for Localized

Immunosuppression. Talk presented at the Society for Biomaterials Annual Meeting; 2018 April 11-14; Atlanta, GA.

Lellouch AG, Taveau CB, Andrews AR, Molde J, Ng ZY, Tratnig-Frankl P, Rosales IA, Goutard M, Lupon E. Lantieri L, Colvin RB, Randolph MA, Kohn J, Cetrulo CL, "Local FK506 Implant Technology in VCA – Successful Bridge to Delayed Mixed Chimerism" (submitted in PRS)

Molde J, Lellouch AG, Steele JAM, Iovine C, Merolli A, Ng ZY, Curtis J. Cetrulo, Jr., Joachim Kohn Engineering Biodegradable Polymeric Devices for Localized Immunosuppression (in progress)

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

Table 2: Personnel efforts and person month worked.

Massachusetts General Hospital

Name	Project Role	Person month worked	Contribution to the project
Curtis Cetrulo	PI	0.36	Overall design and direction of proposed studies, interpretation of results
Alex Lellouch	Research Fellow	1.4	Assist in surgical procedures, analyses of immune responses, interpretation of results
Alec Andrews	Research Technologist	4	Assistance in pre/post operative animal care and overall study coordination

Rutgers University

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	0.30	Grant management
Sanjeeva Murthy	Scientist	0.12	Worked on Polymer design, synthesis & experimental design

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

Current Support Changes for the PI, Co-I or Other Senior/Key Personnel Changes in Current Support	
Curtis Cetrulo	Please see Dr. Cetrulo's current list of other support attached.
Joachim Kohn	Change: Completed DoD grant W81XWH-15-C-0043 "IND Filing for Intravenous cP12 and Pre-IND Studies of Intravenous and Topical cNP5 to Limit Burn Injury Progression" Role: PI Effort: 1.5% Date: 09/24/15-03/31/20 No impact
Joachim Kohn	Change: Completed NSF/DMR grant 1608072 "New Polymeric Biomaterials Inks for 3D Printing" Role: PI Effort: N/A Date: 08/15/16-07/31/20 No impact
Joachim Kohn	Change: Completed Rutgers Techadvance award "Discovery and Validation of Polymers meeting Targeted Product Profile Specifications" Role: PI Effort: N/A Date: 12/16/18-12/05/19 No impact

What other organizations were involved as partners?
Nothing to report.

8. SPECIAL REPORTING REQUIREMENTS

Nothing to report.

QUAD CHARTS: Updated and submitted as attachment.

PREVIOUS, CURRENT AND PENDING SUPPORT – Curtis L. Cetrulo, Jr., M.D.

PREVIOUS (last 5 years)

Award Number: N/A - Established Investigator Grant (Cetrulo)
Funding Agency: Musculoskeletal Transplant Foundation
Title: Costimulation Blockade-Based Regimens of Mixed Chimerism to Overcome Split Tolerance in VCA
Project Goals/Aims: The objective of this proposal is to build on our pilot studies by adding co-stimulatory blockade to promote both successful engraftment after donor bone marrow cell infusion to achieve mixed chimerism and thus tolerance of VCA, and negate split tolerance (i.e. acceptance of all components of a VCA less the epidermis).
Period of Performance: 08/01/2017-07/31/2020
Total Costs:
Effort: 0.6 calendar
Point of Contact: Ava DeGrose
Role: PI

Award Number: W81XWH-17-1-0454 (Cetrulo)
Funding Agency: US Army Medical Research Acquisition Activity
Title: GalT-KO Porcine Nerve Xenograft for Reconstruction of Large Nerve Gaps
Project Goals: This study will compare functional recovery after nerve gap reconstruction using xenograft vs. autograft with FK506 immunosuppression in a nonhuman primate model.
Specific Aims: 1) To demonstrate the efficacy of GalT-KO porcine nerve xenograft based on functional outcome compared to autograft control, 2) Investigate effect of immunosuppression withdrawal on recovery, 3) Corroborate functional outcome data with elecrophysiological (EP) studies and histological analysis of grafts for regeneration or rejection.
Period of Performance: 09/15/2017-03/15/2020
Point of Contact: Andrea Renner,
Role: PI

Award Number: W81XWH-15-1-0281 (Cetrulo)
Funding Agency: US Army Medical Research Acquisition Activity
Title: Local Tacrolimus (FK506) Delivery for Prevention of Acute Rejection in the Non-Human Primate Delayed Mixed Chimerism Vascularized Composite Allograft Tolerance Induction Protocol
Project Goals: We will 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.
Specific Aims: 1) replace systemic tacrolimus and optimize the immunosuppression regime in upper extremity VCA in a non-human primate model, and 2) utilize it as a bridge towards successful tolerance induction by delayed mixed chimerism.
Period of Performance: 09/15/2015-03/15/2020
Point of Contact: Gay Hayden,
Role: PI

Award Number: N/A - Clinical Trial Agreement (Cetrulo)
Funding Agency: AxoGen, Inc.
Title: A Multicenter, Prospective, Randomized, Subject and Evaluator Blinded Comparative Study of Nerve Cuffs and Avance® Nerve Graft Evaluating Recovery Outcomes for the Repair of Nerve Discontinuities (RECON)
Project Goals/Aims: Prospective data collection will be performed to collect injury type, graft utilization, outcome measures and follow-up assessment in subjects who have had peripheral nerve injuries repaired using Avance® Nerve graft and compare to subjects who have had peripheral nerve injuries repaired using nerve cuffs. Data will be compiled by site and by study for analysis.
Period of Performance: 11/25/15-11/24/2019
Point of Contact at Funding Agency: Gillian Robinson, PhD, CCRP
Role: PI

Award Number: N/A - Master Sponsored Research Agreement (Cetrulo)

Funding Agency: XenoTherapeutics, Inc.
Title: MSRA between MGH and XenoTherapeutics
Project Goals/Aims: The goal of this project was to assess the performance of porcine skin graft material after cryopreservation of varying durations.
Period of Performance: 12/15/2016-12/14/2018
Point of Contact: Paul Holzer; XenoTherapeutics
Role: PI

Award Number: N/A - Sponsored Research Agreement (Cetrulo)
Funding Agency: Shire Human Genetic Therapies, Inc.
Title: *SRA between MGH and Shire HGT*
Project Goals/Aims: The goal of this project was to develop a treatment for burn injury by preventing acute inflammation resulting from complement activation using the minipig model of burn injury developed at MGH and demonstrated by Dr. Cetrulo
Period of Performance: 11/1/2016-11/01/2018
Point of Contact: Madhu Natarajan, PhD; Shire
Role: PI

Award Number: 85230-BOS-14 (Cetrulo)
Funding Agency: Shriners Hospitals for Children (SHC) – Boston
Title: Immunology of Hand and Face Transplantation for Burns
Project Goals/Aims: The overall goal of this proposal was to elucidate the mechanisms of vascularized composite allograft (VCA) tolerance (the acceptance of transplanted tissues or organs without rejection in the absence of long-term immunosuppression) in a clinically relevant large animal model, and to contribute to development of a clinical trial-ready protocol. The aims were 1) investigate the contribution of specific cell populations with the donor hematopoietic cell transplant to the induction of mixed chimerism and tolerance of vascularized composite allografts; and 2) investigate the requirement of partial major histocompatibility complex matching on induction of vascularized composite allograft tolerance, immune competence and the risk of graft-versus-host disease.
Period of Performance: 01/01/2014-12/31/2017
Point of Contact: Yong-Ming Yu, MD, PhD; Shriners Hospital for Children – Boston
Role: PI

Award Number: W81XWH-12-2-0037-P00003 (Pomahac)
Funding Agency: DoD/USAMRAA/BWH
Title: A Novel Protocol for Upper Extremity Restoration by Transplantation with Intent for Tolerance Induction
Project Goals/Aims: The aims of this subcontract were to 1) perform upper extremity transplantation followed two months later by delayed bone marrow transplantation in four subjects; 2) determine whether mixed chimerism reduces immune response to UE allografts by in vitro analysis of recipient cell subtypes and function, and to reduce or withdraw immunosuppression; and 3) study the outcomes of UE allotransplantation in a cohort of four patients for a period of one year post-transplant.
Period of Performance: 09/30/2012-09/29/2017
Point of Contact: Elena Howell; USAMRMC
Role: PI (MGH Subaward), Co-Investigator (Overall)

Award Number: W81XWH-13-2-0053 (Atala)
Funding Agency: DoD/Wake Forest (AFIRM II)
Title: Towards a Preclinical Large Animal Tolerance Protocol for Vascularized Composite Allotransplantation in Swine
Project Goals/Aims: This subcontract was to develop a clinically-relevant, mixed chimerism protocol for induction of VCA tolerance across a major histocompatibility barrier in our unique immunophenotyped MGH swine model. The aims were 1) investigate the mechanisms involved in the induction of VCA tolerance in mixed lympho-hematopoietic chimeras; and 2) develop a clinically-relevant, mixed chimerism-based protocol for induction of tolerance of VCA across a major histocompatibility barrier.
Period of Performance: 09/18/2013-09/17/2017
Point of Contact: Linda Mason; Wake Forest
Role: PI (MGH Subaward), Co-Investigator (Overall)

Award Number: W81XWH-13-2-0062 (Cetrulo)
Funding Agency: DoD (RTR)
Title: Tolerance in Nonhuman Primates by Delayed Mixed Chimerism
Project Goals/Aims: The aims of this award were to 1) optimize delayed tolerance induction protocol for VCA in a non-human primate model, 2) investigate the effect of T memory cell inhibition and in vivo T regulatory cells (Treg) up regulation on the delayed induction of VCA tolerance, and 3) investigate the effect of combined Tmem inhibition and Treg up regulation on the delayed induction of VCA tolerance.
Period of Performance: 09/15/2013-09/14/2017
Point of Contact: Mary Alice Woody, PhD; USAMMDA
Role: PI

Award Number: W81XWH-13-2-0060 (Brandacher)
Funding Agency: DoD/Johns Hopkins University (RTR)
Title: Immunomodulation Tolerance Induction after VCA Using Biologic Agents (CTLA4-IG) and Donor BM Cells
Project Goals/Aims: The aims of this project were to 1) establish a belatacept-based protocol to enable CNI minimization after VCA, 2) investigate the possibility to convert from conventional CNI-based immunosuppression to belatacept maintenance with subsequent CNI withdrawal; and 3) compare immunomodulatory donor BM infusion to BM transplantation with establishment of durable mixed chimerism for induction of tolerance and/or VCA survival on CNI free immunosuppression using a belatacept-based regimen.
Period of Performance: 09/15/2013-09/14/2017
Point of Contact: Rochelle Smith; Johns Hopkins
Role: PI (MGH Subaward), Co-Investigator (Overall)

CURRENT

Award Number: W911NF-17-1-0360 (Roth)
Funding Agency: Fred Hutchinson Cancer Research/DoD
Title: Improving Outcome in Ischemia and Ischemia Reperfusion Injury Using Elemental Reducing Agents
Project Goals/Aims: We will test the hypothesis that ERAs improve transplantability of tissue across defined histocompatibility barriers using genetically defined pigs. We aim to determine whether ERAs improve outcome in this model of transplantation.
Period of Performance: 09/01/2017-11/30/2020
Total Costs:
Effort: 0.12 calendar
Point of Contact: Pamela Allen,
Role: PI (MGH Subaward), Co-Investigator (Overall)

Award Number: W81XWH- 16-1-0702 (Cetrulo)
Funding Agency: DoD (RTR)
Title: Optimization of Delayed Tolerance Induction in Swine: A Clinically-Relevant Protocol for Immunosuppression-Free Vascularized Composite Allotransplantation
Project Goals: We will perform VCA transplantation across various genetic barriers to mirror the challenges of finding matching donors in the clinic. This will enable us to determine the extent of genetic matching necessary for clinical VCA success and represents an innovative approach for investigation into the mechanisms involved in rejection and/or acceptance of the VCA.
Specific Aims: The major goals of this project are to 1) modify and apply our previously successful tolerance induction protocol into a clinically-relevant, delayed mixed chimerism approach, and 2) apply this modified delayed tolerance induction approach across various MHC barriers to mirror the clinical challenges of MHC matching for donor-recipient pairs in VCA.
Period of Performance: 09/15/2016-09/14/2020
Total Costs:
Effort: 0.24 calendar
Point of Contact: Lucinda F. Keeney,
Role: PI

Award Number: W81XWH-16-RTRP-TDA (Uygun/Cetrulo)
Funding Agency: Department of Defense, Reconstructive Transplant Research Program

Title: Development of a Supercooled Limb Preservation Protocol
Project Goals: The objective of this application is to adopt our previously successful liver preservation protocol in VCA studies using an established rat hindlimb model to mimic both hand and face transplantation (which consists of skin, muscle, nerve, bone). The specific aims are: 1) to demonstrate the utility of SNMP in resuscitating amputated ischemic limbs following procurement and 2) to incorporate SZNF and SNMP to develop a viable supercooling limb preservation protocol following procurement.
Specific Aims: 1) to demonstrate the utility of SNMP in resuscitating amputated ischemic limbs following procurement, and 2) to incorporate SZNF and SNMP to develop a viable supercooling limb preservation protocol following procurement.
Period of Performance: 09/01/2017-08/31/2020
Total Costs:
Effort: 0.24 calendar
Point of Contact at Funding Agency: Karen L. Petrore,
Role: Co-PI

Award Number: 85103-BOS-18 (Cetrulo)
Funding Agency: Shriners Hospital for Children-Boston
Title: Role of the Thymus in Tolerance of Vascularized Composite Allotransplantation
Project Goals/Aims: The aims of this project are to 1) investigate the requirement of the thymus in establishing VCA tolerance, and 2) to investigate the requirement of the thymus in maintaining VCA tolerance.
Period of Performance: 01/01/2018-12/31/2020
Current Year Direct Costs:
Total Costs: $700,695
Effort: 1.2 calendar
Point of Contact at Funding Agency: Yong-Ming Yu, MD, PhD
Role: PI

Award Number: 85127-BOS-20 (PI: Uygun, B.)
Funding Agency: Shriners Hospital for Children-Boston
Title: Recellularization of vascularized engineered scaffolds for facial reconstruction
Project Goals: This project aims to create complex engineered grafts for facial reconstruction using patient specific cells. If successful, a novel alternative to allografts eliminating the need of immunosuppression will be established.
Specific Aims: Aim 1: Endothelialize decellularized FCF scaffolds, Aim 2: Recellularize FCF scaffolds with skin cells, Aim 3: Test recellularized flaps in vivo.
Period of Performance: 01/01/2020-12/31/2022
Total Costs:
Effort: 0.6 calendar
Point of Contact at Funding Agency: Karen Meader
Role: Co-PI

Award Number: W81XWH1910437 (Cetrulo)
Funding Agency: DoD/USAMRAA
Title: Supercooled Ex-Vivo Porcine VCA preservation to extend the timeline between procurement and transplantation and enable tolerance induction to eliminate immunotherapy needs and risks
Project Goals: The objective of this study is to i) develop an extended subzero non-freezing preservation protocol, which combines oxygenated machine perfusion and supercooling in order to extend storage duration to 3 days and ii) leverage it to implement a tolerance induction protocol for achieving mixed-chimerism based tolerance induction in the graft recipient.
Specific Aims: 1) Scale up of VCA machine perfusion protocol for swine model with transplant validation, 2) Extend preservation duration, and 3) Utilization of sub-zero non-freezing protocol for tolerance induction in swine.
Period of Performance: 08/01/19-07/31/22
Total Costs:
Effort: 1.2 calendar
Point of Contact at Funding Agency: Jason Kuhns, 301-619-1861
Role: PI

Award Number: W81XWH159001 (Cetrulo)
Funding Agency: Medical Technology Enterprise Consortium (MTEC)/Clear Scientific, Inc.
Title: Novel cell-based Therapy to Treat Muscle Atrophy Associated with Peripheral Nerve Injury
Project Goals/Aims: The objective of this study is to develop a cell-based therapy that maintains the capacity of the denervated muscle for synaptic reformation/re-innervation.
Period of Performance: 07/30/19-01/31/21
Total Costs:
Effort: 0.48 calendar
Point of Contact at Funding Agency: Philip Graf, 617-621-8500
Role: PI

PENDING

Award Number: TBD (Farquharson)
Funding Agency: DoD/Real-Time Analyzers, Inc.
Title: Immunosuppressant Drug Monitor
Project Goals/Aims: Transplant patient saliva and blood samples will be collected by MGH. This contract includes the collection of these samples, as well as the analysis by GC-M/MS of the blood samples to validate the RTA measurements of the same using the prototype.
Period of Performance: 06/01/21-11/30/21
Total Costs:
Effort: 0.6 calendar
Point of Contact at Funding Agency: Sue Farquharson, 860-635-9800
Role: PI (MGH Subaward)

Award Number: TBD (Tintle)
Funding Agency: Department of Defense/CDMRP
Title: Assessing the Comparative and Longitudinal Benefits of Vascularized Composite Allotransplantation of the Hand
Project Goals: Vascularized composite allotransplantation (VCA) of the upper extremity (UE) offers tremendous potential to restore function, sensation, and vital independence to service members and civilians who have experienced amputation. The goal of this project is evaluate psychosocial metrics of hand transplant recipients over time.
Specific Aims: AIM 1: Assess the benefits of UE VCA across emotional, social, physical, and functional domains AIM 2: Explore how psychosocial functioning and QOL change over time for UE VCA recipients AIM 3: Develop a consensus set of psychosocial and QOL outcome variables that can be assessed longitudinally across VCA clinical centers
Period of Performance: 07/01/2020-6/30/2023
Current Year Direct Costs:
Effort: 0.3 calendar
Point of Contact at Funding Agency: Jason D. Kuhns, Grants Officer
Role: Co-Investigator (MGH Subaward)

OVERLAP

None

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

Log Number: **MR141092**

Award Number: **W81XWH-15-1-0281**

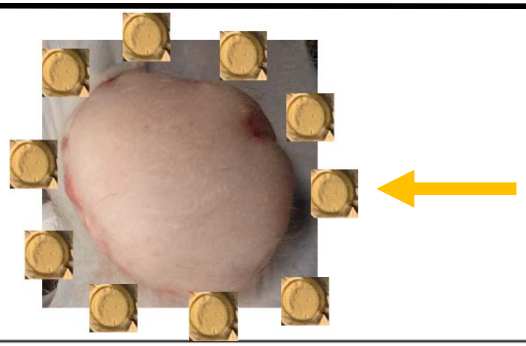
PI: Curtis L. Cetrulo, Jr., M.D., FACS **(Prime) Org**: Massachusetts General Hospital **Award Amount:** $1,042,283

Study Aim(s)

- To develop and characterize Polymeric Local Delivery Systems for tacrolimus and profile the in vivo release kinetics and local tissue distribution of tacrolimus in a small animal model (Rutgers)
- To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free heterotopic partial face allotransplantation in a non human primate (NHP) model
- To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to tolerance induction by delayed mixed chimerism for heterotopic partial face allotransplantation in a NHP model

Approach

By developing an intraoperative, implantable, biomaterials-based, controlled release system for the local administration of tacrolimus into the VCA, the following advantages can be achieved: (1) ensure recipient compliance (2) potential reduction of systemic dose and resultant decrease in risks of systemic side effects and infections (3) localized delivery of the therapeutic at the point of immune cell interaction between donor face and recipient skin immune system. This approach builds upon the preliminary work on device development at Rutgers/NJCB. This technology represents a critical adjunct to the safety profile and efficacy of the delayed tolerance protocol for VCA that is under development at our center at MGH.



Local, skin-specific immunosuppression with subcutaneously placed sustained-release tacrolimus-eluting discs (orange arrow points to schematic representation of subcutaneously placed discs along host-donor suture line).

Accomplishment: Fabrication of FK506-PLDS and in vivo implantation on NHPs

Timeline and Cost

Activities CY	15	16	17	18-20
To develop and characterize Polymeric Local Delivery Systems for Tacrolimus (FK506) and profile the in vivo pharmacokinetics of tacrolimus in a small animal model				
To optimize the tacrolimus implant + systemic immunosuppression dose regimen necessary for rejection-free, infection-free VCA in NHPs				
To provide adjunctive local immunosuppression with the tacrolimus implant as a bridge to delayed tolerance induction of VCA in NHPs				
Summarize optimal immunosuppressive requirements for VCA survival in NHPs. Analyse and summarize data on VCA rejection.				
Estimated Budget ($)	**$3**	**$135**	**$334**	**$569**

Updated: June 14, 2020

Goals/Milestones

CY15 Goal – System demonstration

√ Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS in a small animal model

CY16 Goals – System validation, Production Readiness

√ Demonstrate in vivo safety and profile the in vivo release and local tissue distribution of tacrolimus from PLDS for 2 months.

√ Fabricate and characterize FK506-PLDS for implantation in vivo

CY17 Goal – Product Testing

√ Investigate VCA survival, frequency of rejection and complications, document rejection process clinically and histologically

CY18-19 Goal – Suitability testing

√ Test new tacrolimus disc for group 3 (tolerance induction protocol)

√ Investigate chimerism, in vitro immune status, VCA survival

Comments/Challenges/Issues/Concerns

√ Monitoring of the tacrolimus level

Budget Expenditure to Date

Projected Expenditure: $1,040,743

Actual Expenditure: $1,040,743