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TITLE: Persufflation of composite tissue transplants

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14. ABSTRACT

The objective of this project is to test whether persufflation—perfusion with gaseous oxygen—can extend the time that a composite tissue transplant can be preserved in the cold. This work uses the rat groin flap microsurgical model, in which the femoral vessels serve as the vascular pedicle. Experiments to date have shown that static cold storage in University of Wisconsin organ preservation solution for twenty-four hours is insufficient to maintain the viability of flaps seven days post-implant. Persufflation for twenty-four hours led to better integration of implants, but was not equivalent to unpreserved implants (i.e., immediate replantation). Ongoing studies will optimize the persufflation procedure by varying the persufflation time, the perfusion direction, and the gas pressure.

15. SUBJECT TERMS
Persufflation; oxygen perfusion; cold storage; rat; microsurgery

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1. INTRODUCTION

Composite tissue transplants have shown remarkable results in treating large tissue deficits that result from major traumatic injury. The current standard is to preserve procured tissues in static cold solutions during transport to the operating theater, which limits the storage time to a maximum of 4-6 hours. This project seeks to extend the preservation time by using persufflation—perfusion with gaseous oxygen—as the preservation technique in a rat model of composite tissue transplantation. Persufflation has not previously been applied to composite tissues, although it has shown very promising results in whole organ transplantation. To date, this project has compared the transplantation efficacy of persufflated versus statically stored rat groin flaps. It will optimize the persufflation procedure by varying the gas flow direction, flow pressure and flow intermittency. The targeted preservation time for this work is 24 hours, which would more than triple the current time limit; successful realization of this objective in the rat transplantation model would lay the groundwork for future large-animal and human studies.

2. KEYWORDS

Persufflation; oxygen perfusion; cold storage; rat; microsurgery

3. ACCOMPLISHMENTS

What were the major goals of the project?

The project has two major goals and three milestones, as detailed in the Statement of Work:

1) To compare the efficacy of static cold storage versus persufflation for preservation of composite, adipomusculocutaneous rat groin flaps.

Milestone: Development of procedures for removal, cold storage, persufflation, and transplantation of flap tissue.
Target date: Month 2
Status: Completed

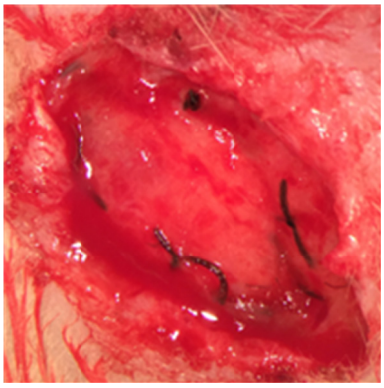
Milestone: Comparison of persufflation versus static cold storage for groin flap transplantation.
Target date: Month 6
Status: Completed

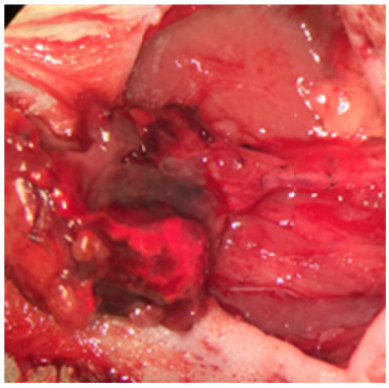
2) To optimize the persufflation technique for 24-hour composite tissue preservation.

Milestone: Determination of optimal persufflation conditions.
Target date: Month 18
Status: 20% completed

What was accomplished under these goals?

The goals of Year 1 were to develop surgical procedures for the removal, cold storage, persufflation, and transplantation of flap tissue, and to compare the effectiveness of persufflation versus static cold storage for flap transplantation. These goals have been accomplished. We are now able to routinely remove, preserve, and transplant flaps on the femoral arteries from one Lewis (inbred) rat to another. These microsurgical implants have shown that unoptimized persufflation was able to partially preserve flaps for twenty-four hours in the cold (4°C). Nevertheless, implants of persufflated flaps did not yield results equivalent to control surgeries (i.e., immediate replantation). Immediate replants led to healthy integration of flaps (*left image*). Flaps that were stored in static University of Wisconsin solution for twenty-four hours led to poor survival seven days after implantation; in particular, thrombosis of the femoral pedicle was apparent (*middle image*). Flaps that were continuously persufflated (i.e., perfused with gaseous oxygen) during cold storage for twenty-four hours appeared to integrate well into surrounding tissue seven days after implantation, although regions of local hematoma were apparent (*right image*). These results suggest that while persufflation may be advantageous over static cold storage for flap preservation, it remains to be optimized.


Control replant (no storage)


Static cold storage (24 hrs)


Persufflation (24 hrs)

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

This work has provided training and professional development for two personnel: Jing Xu and John Jiang. Both served as microsurgeons for this work. Mr. Xu used the project to deepen his surgical experience, which led to his successful application to medical school (with a focus on vascular surgery) in 2018. Mr. Jiang learned the techniques of microsurgical anastomosis in part from Mr. Xu and from the PI, and continues on the project.

How were the results disseminated to communities of interest?

We intend to publish the results of this work next year (2019). To date, the results have not been disseminated publicly.

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

In the next reporting period (Months 13-15), we plan to continue testing different persufflation conditions for use with to-be-transplanted flaps.

4. IMPACT

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

Nothing to report—we have yet to disseminate the results of this work.

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

As mentioned in the previous quarterly progress reports, we are currently running a few months behind schedule, mainly due to the initial slow pace of establishing a surgical routine. Aside from this issue, the work appears to be progressing as planned.

6. PRODUCTS, INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

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

What individuals have worked on the project?

Name: Joe Tien
Project Role: Principal Investigator
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 2
Contribution to Project: Dr. Tien oversees the project and has worked with Jing Xu and John Jiang to establish the surgical procedures.

Name: Jing Xu
Project Role: Surgeon (animal microsurgery)
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 0
Contribution to Project: Mr. Xu has worked with the PI to establish the surgical procedures.

Name: John Jiang
Project Role: Surgeon (animal microsurgery)
Researcher Identifier (e.g. ORCID ID): n/a
Nearest person month worked: 1
Contribution to Project: Mr. Jiang performs the isolation, preservation, and transplantation of the tissues.

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

A quad chart is attached.

9. APPENDICES—None.

Persufflation of Composite Tissue Transplants

RT160128
W81XWH-17-1-0571

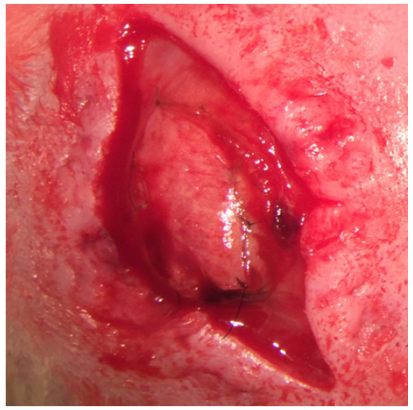
PI: Joe Tien **Org:** Boston University **Award Amount:** $200,000

Study/Product Aim(s)

The overall aim of this project is to investigate the feasibility of using persufflation (perfusion with oxygen gas) for 24-hour hypothermic preservation of composite tissues for subsequent transplantation.

Approach

This work will use the rat groin flap as a model composite tissue. Flaps will be isolated, preserved by persufflation or by static cold storage (the current standard), and transplanted via microsurgical vascular anastomosis. Persufflation protocols will be optimized to improve the functionality of transplanted tissues.



Accomplishments: (*Left*) Transplanted rat groin flap, which was persufflated for 24 hours before replantation. (*Right*) Similarly transplanted flap, but which was persufflated for only 2 hours before replantation. All replants were performed with microsurgical anastomosis on the femoral vessels and evaluated 7 days after implantation. Tissue integration is superior in the 2-hour persufflated flap.

Timeline and Cost

Activities CY	17	18	19
Develop surgical procedures			
Compare persufflation and cold storage			
Optimize persufflation			
Estimated Budget ($K)	**$10**	**$135**	**$55**

Updated: November 12, 2018

Goals/Milestones (Example)

CY17 Goal – Compare efficacy of persufflation vs. static cold storage

☑ Develop surgical procedures (complete)

CY18 Goals – Compare efficacy of persufflation vs. static cold storage; optimize persufflation

☑ Compare persufflation (unoptimized) and static cold storage (complete)

☐Optimize persufflation vessel and pressure

CY19 Goal – Optimize persufflation

☑ Optimize persufflation duration (ongoing)

Comments/Challenges/Issues/Concerns

- The project is roughly three months behind schedule, mainly because surgical procedures have taken longer than expected to establish.

Budget Expenditure to Date

Projected Expenditure: $123k

Actual Expenditure: $65k