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CONTRACTING ORGANIZATION: Metis Foundation, San Antonio, TX

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13. SUPPLEMENTARY NOTES

14. ABSTRACT

Objective:
The objective of this study is to develop an anti-fibrotic therapy using pirfenidone (PFD)-loaded nanostructures to treat proliferative vitreoretinopathy (PVR), a specific type of intraocular fibrosis, in a rabbit model of penetrating eye injury.

Background:
Intraocular fibrosis after penetrating eye injury is a leading cause of vision loss among members of the armed forces, who are vulnerable to penetrating eye injuries due to exposure to high velocity projectiles from detonations of IEDs. Current management of PVR and intraocular fibrosis involves the surgical removal of the intraocular fibrotic tissues. However, despite the 90% anatomic surgical success rate, membranes frequently regrow causing retinal detachment and vision loss, which makes PVR difficult to treat. PFD is a promising pharmaceutical agent approved for the treatment of other types of fibrosis, including pulmonary and renal fibrosis. Although PFD has been shown recently as a possible treatment for intraocular fibrosis, there are concerns associated with its dosage and number of injections. Thus, to develop PFD as a clinical therapy for PVR, the delivery of the drug must be engineered to improve its bioavailability and delivery to the posterior segment of the eye. Success of this study will be a significant advancement not only for the treatment of PVR, but also for the treatment of other disorders that are localized to the posterior ocular segment.

Hypothesis:
We hypothesize that intravitreally injectable PLGA nanoparticles or PU nanocapsules will provide an effective platform for delivery of PFD to the posterior ocular segment for the prevention of intraocular fibrosis in rabbits following penetrating eye injury.

Specific Aims:
Specific Aim 1: Development, characterization, and optimization of PLGA nanoparticles and PU nanoparticles for sustained delivery of PFD to the posterior segment of the eye.
Specific Aim 2: Evaluation of PFD-loaded PLGA nanoparticles and PU nanocapsules *in vitro* for determination of biocompatibility and pharmacodynamics of release.
Specific Aim 3: Assessment of *in vivo* safety, pharmacokinetics, and bioefficacy of intravitreally injected pirfenidone-loaded PLGA nanoparticles and PU nanocapsules.

Study Design:
Aim 1: PFD will be loaded into PLGA nanoparticles and PU nanocapsules, which will be evaluated for their chemical and physical properties, as well as release kinetics.
Aim 2: Retinal pigment epithelial cells will be treated with PFD-loaded nanoparticles and nanocapsules to evaluate their anti-fibrotic functions and cytotoxicity.
Aim 3: Rabbits with penetrating eye injury will receive intravitreal injections of PFD-loaded nanoparticles and nanocapsules. The nanostructures will be tracked to ensure delivery to the posterior segment. The rabbit eyes will be scored for severity of intraocular fibrosis in treated versus untreated groups.

15. SUBJECT TERMS None listed.

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON** USAMRDC
a. REPORT Unclassified	**b. ABSTRACT** Unclassified	**c. THIS PAGE** Unclassified	Unclassified	14	**19b. TELEPHONE NUMBER** *(include area code)*

Table of Contents

1. INTRODUCTION

Posterior penetrating eye injury is a common battlefield-related ocular injury. Improper retinal wound healing can result in intraocular fibrosis and lead to severe visual impairment. Although there have been numerous technical advances in the surgical management of intraocular fibrosis, the continuous scarring in the eye after surgical intervention can lead to severe visual impairment or blindness. This suggests that a pharmacological treatment is needed. Pirfenidone (PFD) is a promising anti-scarring agent that has been approved for the treatment of pulmonary fibrosis and has also shown great potential in the prevention of liver or renal fibrosis. The objective of this study is to develop novel nanostructures for sustained delivery of PFD to the posterior segment of the eye. The therapeutic effects of PFD-loaded nanoparticles or nanocapsules for the prevention and treatment of retinal scarring will be validated using a rabbit model of posterior penetrating eye injury.

2. KEYWORDS

Proliferative Vitreoretinopathy (PVR), Intraocular Fibrosis, Pirfenidone (PFD), poly lactic-co-glycolic acid (PLGA) Nanoparticles, Polyurethane (PU) Nanocapsules, Posterior Penetrating Eye Injury

3. ACCOMPLISHMENTS

What were the major goals of the project? (Goals to be accomplished and status.)

Navy Medical Research Unit San Antonio (NMRU-SA) Updates:

The long-term goal of this project is to develop novel nanostructures for the sustained delivery of therapeutic agents to treat PVR. The project objectives are 1) to develop biodegradable nanoparticles and nanocapsules to improve the delivery of PFD in the ocular environment, and 2) to achieve delivery of PFD to the posterior segment of the eye in a rabbit penetrating eye injury model. Studies outlined in the following three specific aims will be completed to address the stated project objectives:

Specific Aim 1: Development, characterization, and optimization of PLGA nanoparticles and PU nanocapsules for the delivery of PFD to the posterior segment of the eye.

STATUS: Completed. Synthesis and characterization of two types of PFD-loaded PLGA-pluronic nanoparticles (PLGA-PF68 and PLGA-PF127) and PFD-loaded PU nanocapsules have been completed and published.

Specific Aim 2: Evaluation of PFD-loaded PLGA nanoparticles and PU nanocapsules *in vitro* for determination of biocompatibility and pharmacodynamics of release.

STATUS: Completed. We performed *in vitro* safety and biocompatibility studies on both PLGA nanoparticles and PU nanocapsules using ARPE-19 cells. The bioefficacy of PFD-loaded PU nanocapsules was evaluated using NIH3T3 cells (published). Additional experiments on free PFD were conducted to analyze the secretion profiles of iPS-RPE cells in response to PFD during wound healing (manuscript under revision).

Specific Aim 3: Assessment of *in vivo* safety, pharmacokinetics, and bioefficacy of intravitreally injected PFD-loaded PU nanocapsules in a penetrating eye injury rabbit model.

STATUS: Ongoing. We finished data collection and analysis for Experiment 1: *In Vivo* Pharmacokinetics and Distribution of PFD-loaded Nanocapsules. Preparations are underway for Experiment 2: Investigation of Anti-fibrotic Effects and Visual Function Preservation of PFD-loaded PU Nanocapsules in Rabbits with Posterior Penetrating Eye Injury.

What was accomplished under these goals? (Detailed progress and results.)

Navy Medical Research Unit San Antonio (NMRU-SA) Updates:

Specific Aim 1: Development, characterization, and optimization of PLGA nanoparticles and PU nanocapsules for the delivery of PFD to the posterior segment of the eye.

Key Findings or Accomplishments:

PLGA Nanoparticles: We have successfully synthesized two types of PFD-loaded PLGA-pluronic nanoparticles (PLGA-PF68 and PLGA-PF127) using a modified nanoprecipitation method. The morphology of

PLGA nanoparticles was found to be spherical based on scanning electron microscopy analysis. Size distribution and zeta potential (ZP) of the nanoparticles were analyzed using a Zetasizer Nano ZS90 instrument (Spectris/Malvern Panalytical, Malvern, UK). Particle size of PLGA-PF68 and PLGA-PF127 nanoparticles ranged from 262.3±21.08 to 548±19.3nm in water, and from 239.86±6.8 to 972.8±24.94nm in DMEM, whereas ZP of the nanoparticles ranged from -36.33±1.3 to -11.33±0.35mV in water, and from -34.63±0.8 to -1.29±0.15mV in DMEM. A sustained release of PFD has been observed in PLGA PF68 and PLGA PF127 nanoparticles for up to 90 days.

PU Nanocapsules: We have successfully synthesized PU nanocapsules encapsulated with PFD via interfacial polymerization. The morphology of these nanostructures was characterized using transmission electron microscopy and was found to be spherical. The PFD-loaded PU nanocapsules had an average size of 244.666±40.06nm in ethanol, 836±12.8nm in DMEM, and 579±54.1nm in water. ZP of nanocapsules was -50.44±11.72mV in KCl, -24.1±1.8mV in DMEM, and -37.36±0.47mV in water. PU nanocapsules appeared to disperse very well in ethanol, but agglomerate in DMEM. Greater ZP values were observed in KCl and water than DMEM. We have also completed release kinetics studies on PFD-loaded PU nanocapsules, where a sustained release of the drug was observed under sink conditions for up to 150 days.

Specific Aim 2: Evaluation of PFD-loaded PLGA nanoparticles and PU nanocapsules *in vitro* for determination of biocompatibility and pharmacodynamics of release.

Key Findings or Accomplishments:

We have completed *in vitro* safety and biocompatibility studies on PLGA nanoparticles and PU nanocapsules using human RPE (ARPE-19) cells. The MTS assay was used to quantify the number of viable cells after a 24hr incubation with 0-500μg/mL of PLGA PF68 or PLGA PF127 nanoparticles or with 0-1000μg/mL of PU nanocapsules. Oxidative stress or reactive oxygen species activity in PU nanocapsule-treated RPE cells was measured using the DCFDA assay and was found to be below baseline levels. Overall, we have shown that PLGA nanoparticles and PU nanocapsules were not cytotoxic and presented no adverse effects on RPE cell viability.

The bioactivity of PFD released from PU nanocapsules was assessed using a fibroblast proliferation and migration assay. Supernatants of PFD-loaded PU nanocapsules from three different time points (*in vitro* release at 1 hr, 1 day and 1 week) were added to wounded NIH3T3 cells. At 24 hrs post-wounding, the gaps associated with the control group were approximately 10% of the gaps seen at 0 hrs. In contrast, all the supernatants from the PU release study had identical gaps that were 55-60% of the gaps seen at 0 hrs, a clear indication that the released PFD was bioactive.

We also conducted an *in vitro* wound healing assay on human iPS-RPE cells using free PFD. iPS-RPE cells were grown on transwell inserts until fully confluent and pigmented, then scratched using a pipet tip to induce a rectangular wound. Cell monolayers were cultured in complete media (CM), CM plus TGFβ2, CM plus TGFβ2 and PFD. Cell proliferation and migration into the wound gap area, along with phenotypic changes of the cells in response to wounding were examined. Moreover, media from apical and basolateral chambers were collected prior to wounding on Day 0 and every 48 to 72 hours post-wounding for up to 14 days.

A selection of 16 cytokine/growth factor targets that have been implicated in PVR, EMT, and iPS-RPE wound healing were analyzed via multiplex ELISA for this study. These targets included fibroblast growth factor-2 (FGF-2), granulocyte-macrophage colony-stimulating factor (GM-CSF), growth-regulated oncogene α (GROα), interleukin-1 α (IL-1α), interleukin-1 β (IL-1β), interleukin-6 (IL-6), interleukin-8 (IL-8), interleukin-17A (IL-17A), *monocyte chemoattractant protein-1 (*MCP-1), platelet-derived growth factor-AA (PDGF-AA), platelet-derived growth factor-BB (PDGF-BB), tumor necrosis factor α (TNFα), endothelial growth factor-A (VEGF-A), in addition to 3 isoforms of TGFβs (TGFβ1, 2 and 3). Five targets including FGF-2, GM-CSF, IL-1β, IL-17A, PDGF-BB fell below the detection range and were excluded from further analysis.

Most factors such as TNFα, IL-1α, MCP-1, and PDGF-AA exhibited polarized (apical-basal) secretion patterns, whereas a few factors (e.g., TGFβ1 and TGFβ3) showed similar levels in apical and basolateral media (see **Table 1**). The goal of this study was to evaluate the impact of PFD on the secretion of soluble factors that may impede EMT or wound healing. Overall, the expression of growth factors/cytokines typically associated with EMT was elevated in the presence of TGFβ2, but was reduced with the addition of PFD. We believe that the iPS-RPE response to injury resulted from a synergistic effect of many factors that worked in concert to activate RPE cells, initiate EMT, and promote wound healing. Similarly, the inhibitory role of PFD was likely achieved through a synergistic effect of a multitude of growth factors and cytokines.

Table 1: Growth factor/cytokine secretion profile of iPS-RPE during wound healing.

	Apical		Basolateral		Polarity
	Ctr vs. TGFβ2	TGFβ2 vs. TGFβ2+PFD	Ctr vs. TGFβ2	TGFβ2 vs. TGFβ2+PFD	
TGFβ1	*		*		No
TGFβ2		***		*	Yes (Higher Apical)
TGFβ3					No
TNFα				*	Yes (Higher Apical)
IL-1α	*	*			Yes (Higher Apical)
IL-6	**	**	**	**	Yes (Higher Apical)
IL-8					Yes (Higher Apical)
GROα	***	*	*		Yes (Higher Apical)
MCP-1	**	**			Yes (Higher Apical)
PDGF-AA	**	**		**	Yes (Higher Apical)

Specific Aim 3: Assessment of *in vivo* safety, pharmacokinetics, and bioefficacy of intravitreally injected PFD-loaded PU nanocapsules in a rabbit penetrating eye injury model.

Key Findings or Accomplishments:

We received IACUC approval for our animal protocol entitled "Development of Nanocapsule-based Anti-fibrotic Therapeutics for Post-Traumatic Proliferative Vitreoretinopathy in the Rabbit (Oryctolagus Cuniculus)" on 10/6/22 and completed Experiment 1 of the animal study to assess the *in vivo* pharmacokinetics and distribution of PFD-loaded PU nanocapsules in an established rabbit penetrating eye injury model.

Fifteen male Dutch Belted rabbits were divided into 3 groups (n=5 per time point). Only the right eye of each animal was subjected to a penetrating eye injury. The contralateral eye served as an internal sham/non-injury control. PFD-loaded PU nanocapsules (28.02±13.75 μg PFD/mg PU-PFD nanocapsule) were injected into the eye post-injury. Standard eye assessments included intraocular pressure (IOP), fundus photography and electroretinogram (ERG) as shown in **Figure 1**. All rabbits showed good tolerance of PFD-loaded PU nanocapsules with small variations of IOP and ERG measurements throughout the entire duration of the experiment. Fundus images clearly illustrated the induction of penetration injury and helped to track the progression of the PU-PFD treatment at different time points.

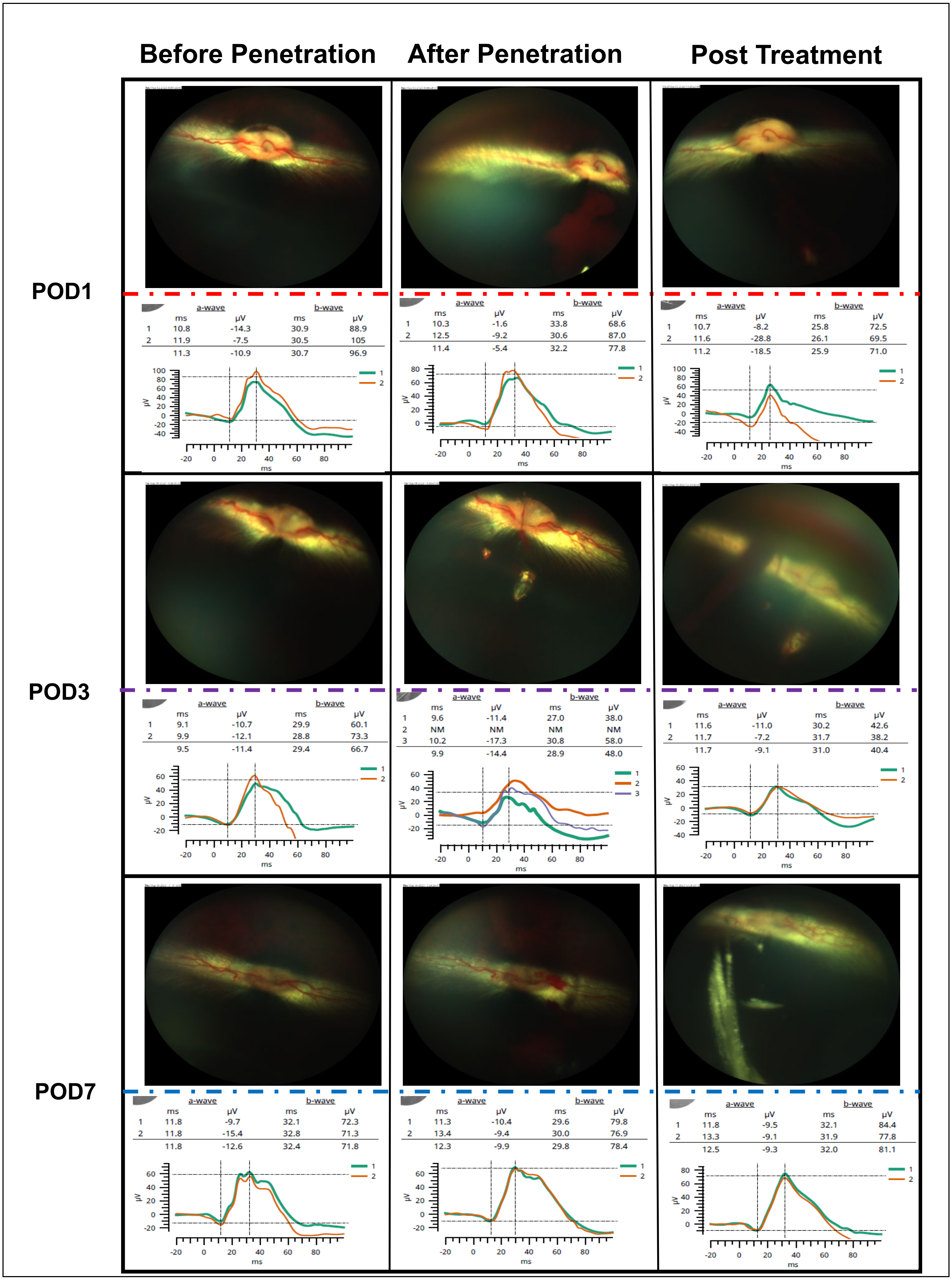

Before Penetration
After Penetration
Post Treatment
POD1
POD3
POD7
a-wave
b-wave
ms μV ms μV
1 10.8 -14.3 30.9 88.9
2 11.9 -7.5 30.5 105
11.3 -10.9 30.7 96.9
1 10.3 -1.6 33.8 68.6
2 12.5 -9.2 30.6 87.0
11.4 -5.4 32.2 77.8
1 10.7 -8.2 25.8 72.5
2 11.6 -28.8 26.1 69.5
11.2 -18.5 25.9 71.0
1 9.1 -10.7 29.9 60.1
2 9.9 -12.1 28.8 73.3
9.5 -11.4 29.4 66.7
1 9.6 -11.4 27.0 38.0
2 NM NM NM NM
3 10.2 -17.3 30.8 58.0
9.9 -14.4 28.9 48.0
1 11.6 -11.0 30.2 42.6
2 11.7 -7.2 31.7 38.2
11.7 -9.1 31.0 40.4
1 11.8 -9.7 32.1 72.3
2 11.8 -15.4 32.8 71.3
11.8 -12.6 32.4 71.8
1 11.3 -10.4 29.6 79.8
2 13.4 -9.4 30.0 76.9
12.3 -9.9 29.8 78.4
1 11.8 -9.5 32.1 84.4
2 13.3 -9.1 31.9 77.8
12.5 -9.3 32.0 81.1
μV
ms

Figure 1: **Representative fundus images and ERG measurements of three rabbit eyes before or after penetration injury, immediately after treatment, and post-treatment on POD1, POD3 and POD7.**

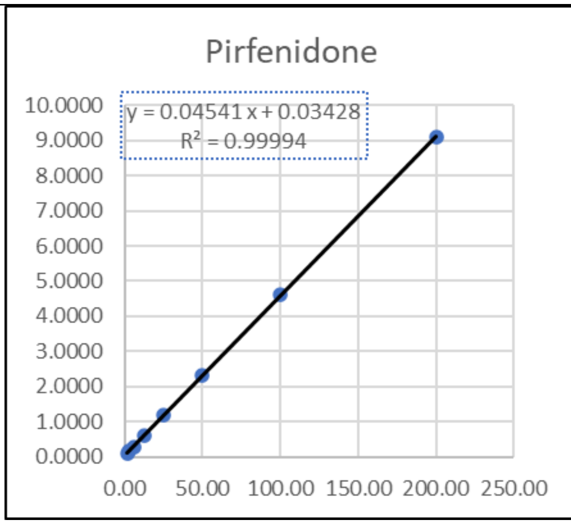
Rabbit eyes were enucleated and stored at -80°C following post-treatment assessments on POD1, 3 and 7. PFD was extracted from the vitreous and retina and quantified using high-performance liquid chromatography - mass spectrometry (HPLC-MS). Briefly, whole eye specimens were removed from the freezer and cut through the cornea and choroid. While thawing on ice, the cornea and choroid were peeled off and discarded. The retina and vitreous humor of each sample were separated and placed in Eppendorf tubes. 0.5mL of methanol was added to each tube, homogenized, washed, and centrifuged at 12000rpm for 10 min. The resultant supernatant was adjusted in volume with additional methanol and divided into two equal aliquots. One sample from each time point (POD1, 3 and 7) was selected and further prepared for HPLC-MS analysis using an AB SCIEX 5500 mass spectrometer.

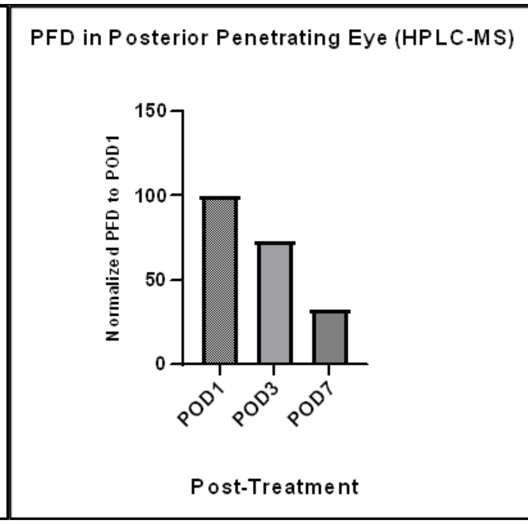
Table 2 shows the raw data for HPLC-MS. S1-S8 correspond to the standard curve, whereas POD1, POD3, POD7 represent selected samples from the three time points. Free PFD with a known concentration of 1.5mg/mL served as the positive control. POD1, POD3, POD7 samples were diluted 16-fold, whereas the positive control was diluted 320-fold. The analyzed concentration of the positive control was 1.63mg/mL (vs. 1.5mg/mL known concentration), which reflected the accuracy of the analysis. Carbamazepine was used as the internal standard.

Table 2: **PFD HPLC-MS raw data**

Content (mg/mL) (320x dilution)	Content (ug/mL) (16x dilution)	Standard (S1-8) Sample (POD1,3,7)	Conc. (ng/mL)	Area (Carbamazepine)	Area (Pirfenidone)	Ratio (Pirfenidone/Carbamazepine)
		S1	1.56	4.80E+05	4.39E+04	0.0915
		S2	3.13	4.98E+05	8.33E+04	0.1673
		S3	6.25	5.24E+05	1.55E+05	0.2958
		S4	12.5	4.72E+05	2.83E+05	0.5996
		S5	25.0	4.22E+05	5.00E+05	1.1848
		S6	50.0	4.28E+05	9.93E+05	2.3201
		S7	100	3.94E+05	1.82E+06	4.6193
		S8	200	3.52E+05	3.20E+06	9.0909
	1.76	POD1	110.25	3.69E+05	1.86E+06	5.041
	1.29	POD3	80.51	3.55E+05	1.31E+06	3.690
	0.58	POD7	36.12	3.93E+05	6.58E+05	1.674
1.63		Positive Control	5079.19	8.67E+04	2.00E+07	230.681

A standard curve was constructed by plotting the area ratio of PFD to Carbamazepine as a function of concentration. The standard curve was linear, and the equation relating to the area of peaks (Y) against concentration of PFD (X) was Y=0.04541X + 0.03428 and R^2= 0.99994 as shown in **Figure 2**.





<u>Figure 2</u>: Standard curve used to determine the concentration of PFD (left panel). Relative amount of PFD when normalized to POD1 (right panel).

Since 3mg of PFD-loaded PU nanocapsules were used to deliver PFD and there were 28.02±13.75μg of PFD per mg of PU-PFD nanocapsule, the total amount of PFD administered was around 84±41μg. The amount of PFD remained on POD1, 3 and 7 was ~3.53μg, 2.58μg and 1.16μg, respectively. **Figure 2** shows a plot of the relative amount of PFD when normalized to POD1. The amount of PFD remained on POD7 was 33% of the PFD on POD1, whereas the amount of PFD detected on POD3 was 73% of the PFD on POD1.

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

Nothing to report.

How were the results disseminated to communities of interest?

Navy Medical Research Unit San Antonio (NMRU-SA) Updates: We published a manuscript on "Polyester Nanoparticles and Polyurethane Nanocapsules Deliver Pirfenidone to Reduce Fibrosis and Scarring" in ACS Biomater Sci Eng on May 17, 2023. We presented a poster entitled "Anti-Fibrotic Effects of Pirfenidone on Human iPS-derived Retinal Pigment Epithelial Cells in an In Vitro Model of Proliferative Vitreoretinopathy" at the Association for Research in Vision and Ophthalmology (ARVO) annual meeting on April 23-27, 2023, in New Orleans, LA. We also presented a poster on "Pirfenidone-loaded Polyurethane Nanocapsules Reduce Fibrosis and Scarring" at the Military Health System Research Symposium (MHSRS) annual meeting on August 14-17, 2023, in Kissimmee, FL.

Plans for the next reporting period to accomplish the goals

Navy Medical Research Unit San Antonio (NMRU-SA) Updates: We plan to complete the *in vivo* studies on the investigation of anti-fibrotic effects and visual function preservation of PFD-loaded PU nanocapsules in rabbits with posterior penetrating eye injury.

4. IMPACT

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

Navy Medical Research Unit San Antonio (NMRU-SA) Updates: Upon completion of the proposed research, invaluable data will be generated in the development of novel PLGA nanoparticles and PU nanocapsules that can enhance the bioavailability of PFD, facilitate its delivery to the posterior segment of the eye, and reduce fibrosis in rabbits with penetrating eye injury. The impact of these studies will be significant for several reasons. As of now, an effective treatment for PVR is lacking. Even with surgical intervention, the visual outcomes for patients are poor, leading to severe visual impairment or blindness. Furthermore, an effective system for delivering pharmaceutical agents to the posterior segment is not currently available.

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**

IMPORTANT REMINDER – Award recipient organization is required to obtain prior written approval from the awarding agency Contracting/Grants Officer whenever there are significant changes in the project or its direction such as significant change in scope or the Statement of Work (e.g. removal, change, or addition of aims/tasks or animal model change), change in PI or key personnel, reduction of 25% FTE, or significant change in budget.

Changes in approach and reasons for change

Navy Medical Research Unit San Antonio (NMRU-SA) Updates: As stated in the proposal, we had originally planned to develop functionalized Avidin Fatty Acid (AFA)-PLGA nanoparticles for the delivery of PFD to the posterior segment of the eye. However, after several preliminary trials, it became clear that AFA-PLGA nanoparticles were unstable and may not deliver the drug efficiently. As a result, we modified our formulations with pluronics to encapsulate PFD and to overcome the issues with instability. To date, we have successfully synthesized two types of PFD-loaded PLGA-pluronic nanoparticles (PLGA-PF68 and PLGA-PF127), in addition to PFD-loaded PU nanocapsules. Since PU nanocapsules can be trigged for on-demand drug release and possess other characteristics that are highly favorable for ocular drug delivery, we shifted our focus to PFD-loaded PU nanocapsules for the *in vivo* study, where we administered the nanocapsules via intravitreal injection to the posterior segment of the eye.

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

Navy Medical Research Unit San Antonio (NMRU-SA) Updates: The COVID-19 crisis has caused significant delays in the progress of this project. Despite our continuous efforts, we were not able to finish all the animal studies by the end of this fiscal year. Therefore, we have submitted a no-cost extension for the purpose of completing Experiment 2 of the *in vivo* work as specified in Specific Aim 3.

Changes that had a significant impact on expenditures

Nothing to report.

Significant changes in use or care of human subjects

Not applicable.

Significant changes in use or care of vertebrate animals

Navy Medical Research Unit San Antonio/NMRU Updates:

TOTAL PROTOCOL(S): 1

PROTOCOL (1 of 1 total):
IACUC Protocol Number: Navy-22-19

ACURO Protocol Number: N/A
Protocol PI: Heuy-Ching Wang, Ph.D.
Protocol Site: Naval Research Unit San Antonio (NAMRU-SA), Tri-Service Research Laboratory (TSRL)
Protocol Title: Development of Nanocapsule-based Anti-fibrotic Therapeutics for Post-Traumatic Proliferative Vitreoretinopathy in the Rabbit (Oryctolagus Cuniculus)

Number of Animals Approved for Use: 66
IACUC INITIAL APPROVAL DATE: 10/6/2022 (expires 10/6/2025)
ACURO INITIAL APPROVAL DATE: N/A
RENEWAL APPROVAL DATES:
- TBD

AMENDMENTS:
- None.

ADVERSE EVENTS OR UNANTICIPATED PROBLEMS:
- None.

Significant changes in use of biohazards and/or select agents

Not applicable.

6. PRODUCTS

Journal publications

1. Liu S, Ale T, Kehinde V, Ale T, **Wang H**, Lavik E. Polyester Nanoparticles and Polyurethane Nanocapsules Deliver Pirfenidone to Reduce Fibrosis and Scarring. ACS Biomater Sci Eng. 2023 May 17. doi: 10.1021/acsbiomaterials.3c00087. Online ahead of print.
    a. Original manuscript
    b. Published
    c. Directly related to SOW, Specific Aim 1
    d. DoD funding acknowledged.
2. Blackford BG, Justin GA, Baker KM, Brooks DI, **Wang HC**, Ryan DS, Weichel ED, Colyer MH. Proliferative Vitreoretinopathy After Combat Ocular Trauma in Operation Iraqi Freedom and Operation Enduring Freedom: 2001-2011. Ophthalmic Surg Lasers Imaging Retina. 2020 Oct 1;51(10):556-563.
    a. Original manuscript
    b. Published
    c. Directly related to SOW, Specific Aim 3
    d. DoD funding acknowledged.
3. Greene W, Burke T, Bramblett G, **Wang HC**. Detection of Retinal Fibrosis in a Rabbit Model of Penetrating Eye Injury. Mil Med. 2020 Jan 7; 185 (Suppl 1): 443-447.
    a. Original manuscript
    b. Published
    c. Directly related to SOW, Specific Aim 3
    d. DoD funding acknowledged
4. Greene WA, Kaini RR, **Wang HC**. Utility of Induced Pluripotent Stem Cell-Derived Retinal Pigment Epithelium for an In Vitro Model of Proliferative Vitreoretinopathy. Adv Exp Med Biol. 2019;1186:33-53.
    a. Original manuscript
    b. Published
    c. Directly related to SOW, Specific Aim 2

d. DoD funding acknowledged.

Books or other non-periodical, one-time publications

Nothing to Report.

Other publications, conference papers, and presentations

5. **Wang HC**, Liu S, Ale T, Kehinde V, Jiang S, Rettinger CL, Rodriguez A, Colyer MH, Lavik E. Pirfenidone-loaded Polyurethane Nanocapsules Reduce Fibrosis and Scarring. MHSRS, August 14-17, 2023
    a. Conference proceeding
    b. Published
    c. Related to SOW, Specific Aim 1
    d. DoD funding acknowledged.
6. **Wang HC**, Jiang S, Rettinger CL, Colyer MH. Anti-Fibrotic Effects of Pirfenidone on Human iPS-derived Retinal Pigment Epithelial Cells in an In Vitro Model of Proliferative Vitreoretinopathy. Investigative Ophthalmology & Visual Science June 2023, Vol.64, 4576.
    a. Conference proceeding
    b. Published
    c. Related to SOW, Specific Aim 3
    d. DoD funding acknowledged.
    e.
7. Mendez JA, Lundquist B, Rettinger, CL, Colyer MH, **Wang HC**. Pirfenidone Inhibits Proliferation, Migration and Fibrosis in an *In Vitro* Model of Proliferative Vitreoretinopathy. MHSRS, September 12-15, 2022, Kissimmee, FL.
    a. Conference poster presentation
    b. Presented
    c. Related to SOW, Specific Aim 2
    d. DoD funding acknowledged.
8. **Wang HC**, Peitzsch A, Martinez L, Tewolde S, Cardin S. Automating the Detection of Retinal Tears from Vascular Deformation using Machine Learning. Investigative Ophthalmology & Visual Science June 2021, Vol.62, 2126.
    a. Conference proceeding
    b. Published
    c. Related to SOW, Specific Aim 3
    d. DoD funding acknowledged.

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?

Naval Medical Research Unit-San Antonio (NMRU-SA)
Name: Dr. Heuy-Ching Wang
Project Role: Principal Investigator
Nearest person month worked: 7.0 months
Contribution to the project: Dr. Wang has been overseeing the proposed project.

University of Maryland, Baltimore County (UMBC)
Name: Dr. Erin Lavik
Project Role: Co-Investigator (no pay)
Nearest person month work: 2.0 months
Contribution to the project: Dr. Lavik is a biomaterial expert and serves as a co-investigator for this project.

Madigan Army Medical Center (MAMC)
Name: Marcus Colyer
Project Role: Consultant (no pay)
Nearest person month work: 2.0 months
Contribution to the project: Dr. Colyer is a vitreoretinal surgeon and an ophthalmology consultant to the Surgeon General of the US Army. Dr. Colyer serves as a consultant for this project.

Metis Foundation
Name: Dr. Christina Rettinger
Project Role: Investigator
Nearest person month work: 7.0 months
Contribution to the project: Dr. Rettinger has written laboratory protocols, animal protocols, technical reports, progress reports, abstracts, and presentations for this project.

Name: Mr. Patrick Hsun
Project Role: Investigator
Nearest person month work: 12 months
Contribution to the project: Mr. Hsun has refined the machine learning algorithm for automated detection of retinal tears from vascular deformation using fundus images of rabbit eyes with penetrating injury.

Name: Dr. Shoulei Jiang
Project Role: Investigator
Nearest person month work: 12.0 months
Contribution to the project: Dr. Jiang joined the group on 8/15/22. He worked on the *in vitro* PVR assay and analyzed the cytokine secretion data. He also helped to execute the *in vivo* study.

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?

1. Organization Name: University of Maryland, Baltimore Country (UMBC)

Location of Organization: Baltimore, MD

Partner's Contribution to the Project: Provided equipment/personnel/facility to manufacture and characterize PLGA nanoparticles and PU nanocapsules as specified in AIM#1. Supplied PU nanocapsules for AIM#2 (*in vitro* work) and AIM#3 (*in vivo* work). Dr. Erin Lavik, PhD, served as a consultant for the nanoparticle/nanocapsule-based work.

2. Organization Name: Madigan Army Medical Center (MAMC)

Location of Organization: Joint Base Lewis-McChord, WA

Partner's Contribution to the Project: Dr. Marcus Colyer, MD, LTC(P), MC, USA, served as a clinical consultant for combat-induced PVR.

8. SPECIAL REPORTING REQUIREMENTS

QUAD CHART

9. APPENDICES