Duquesne University

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2011 – June 30, 2012

Formula Grant Overview

The Duquesne University received $116,091 in formula funds for the grant award period January 1, 2011 through December 31, 2013. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

From Insoluble Perfluorocarbon Oils to Multifunctional Nanoparticles for Breast Cancer Imaging and Treatment - The purpose of this project is the development of novel multifunctional perfluorocarbon (PFC) based magnetic resonance (MR) detectable drug delivery vehicles. Specifically, nanoemulsions, microemulsions and gels for localized delivery of anti-inflammatory agents to breast tumors will be prepared. The PFC based drug delivery vehicle localization, accumulation and distribution can ultimately be quantitatively and qualitatively assessed in vivo by $^{19}$F MRI. Fluorine-19 has very low biological abundance in tissues and $^{19}$F MR directly detects the density of $^{19}$F spins contained in the PFC molecules without background. We hope to develop true theranostic agents, therapeutic and diagnostic for breast tumor imaging and treatment. Recent epidemiological studies demonstrated that treatment with nonsteroidal anti-inflammatory agents (NSAIDs), such as COX2 inhibitors, can reduce the risk of developing breast cancer, with aspirin and celecoxib showing the most significant effects. Clinical and experimental evidence strongly suggest COX2 inhibitors as new adjuvant breast cancer treatments. The purpose of this project is to incorporate a COX2 inhibitor into a $^{19}$F MRI visible nanoreagent for anti-inflammatory adjuvant treatment in breast cancer.

Anticipated Duration of Project

1/1/2011 - 12/31/2013

Project Overview

The focus of this project is the design, synthesis and in vitro evaluation of novel multifunctional perfluorocarbon (PFC) based magnetic resonance (MR) detectable drug delivery vehicles. We will examine synthetic and formulation aspects of several carefully selected PFCs and assess their viability for theranostic, therapeutic and diagnostic reagent development.

Hypothesis: Perfluorocarbons are a highly viable platform for engineering multifunctional nanoreagents for simultaneous breast cancer treatment and imaging. The hypothesis will be
tested by the following specific aims. **Specific Aim 1**: Design and synthesis of perfluorinated amphiphiles, perfluorocarbon-hydrocarbon conjugates and perfluorinated crosslinkers. **Specific Aim 2**: Formulation of chemically modified PFCs into nanoemulsions, microemulsions and gels for localized drug delivery to breast tumors.

**Methods**: Perfluorinated conjugates will be designed and synthesized to incorporate fluorocarbon polymers, simple perfluorocarbon chains \((\text{CF}_2)_n\) or perfluoropolyethers \((\text{CF}_2\text{CF}_2\text{O})_n\), and either a lipophilic or hydrophilic short chain hydrocarbon or polymer. Synthetic protocols will include, but are not limited to, Mitsunobu reactions, click chemistry and activated fluorinated ester conjugations. Trifluoromethyl group(s) will be introduced into lipids, alcohols and surfactants. Perfluoropolyethers will be conjugated to hydrocarbons or crosslinked into gels. Nanoemulsions and microemulsions will be prepared by sonication, microfluidization and low energy emulsification methods. Droplet size, shape and surface properties will be measured by dynamic light scattering (DLS), zeta potential and microscopy. Perfluorinated gels will be synthesized by chemical crosslinking of ionic and non-ionic polymers with perfluorocarbon crosslinking reagents. Gels will be evaluated for rheological properties (e.g., viscosity, elasticity), spreading properties and physical strength. The effects of pH, temperature and ionic strength on drug incorporation and release will be tested in all formulations. \(^{19}\text{F}\) NMR will be used to measure \(^{19}\text{F}\) content, evaluate fluorocarbon-hydrocarbon interactions and assess the potential “imageability” of each vehicle by \(^{19}\text{F}\) MRI.

The focus of our efforts is on the synthesis and formulation of PFC reagents and assessing their viability as drug delivery vehicles, using celecoxib as a model drug, *in vitro*. The attractive PFC drug delivery candidates will be subjected to simple cell culture tests. *In vivo* biological evaluation is beyond the scope of this project.

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Claire E. O’Hanlon, BS, Robert K. Tunney Jr., Sravan Patel, MS - employed by Duquesne University

**Expected Research Outcomes and Benefits**

Each year, 465,000 people die of breast cancer and 1.3 million new cases are diagnosed. One third of women diagnosed with early stage breast cancer will eventually develop metastatic breast cancer. Many women do not seek treatment during the early stage of the disease and are first diagnosed in stage II or III. The goal of this work is to improve breast cancer therapy by
targeting primary tumor-associated inflammation with the hope to decrease its metastatic potential and improve chemotherapy response. Recent epidemiological studies demonstrated that treatment with nonsteroidal anti-inflammatory agents (NSAIDs), such as COX2 inhibitors, can reduce the risk of developing breast cancer, with aspirin and celecoxib showing the most significant effects. Clinical and experimental evidence strongly suggests COX2 inhibitors as new adjuvant breast cancer treatment. However, when applied systemically, COX2 inhibitors can cause potentially life-threatening side effects, such as cardiovascular toxicity and gastrointestinal disturbances. Targeted delivery of COX2 inhibitors to breast tumors and inflammatory peritumoral areas directly may overcome these problems. Celecoxib, a selective COX2 inhibitor, is insoluble in water and has low oral bioavailability (20-60%).

Drug delivery vehicles for poorly soluble anti-inflammatory drugs will be prepared. The PFC component will serve two purposes: 1) provide a biologically inert platform for delivering an anti-inflammatory agent, and 2) serve as a MRI tracer for in vivo monitoring of the drug delivery system efficiency. The PFC based nanoreagent development in this project has the potential to open new avenues for drug delivery and development, potentially leading to new breast cancer treatment approaches. The project is highly collaborative and will strengthen partnerships between Duquesne University, Carnegie Mellon University and Celsense Inc. (Pittsburgh, PA), a biotechnology company, the current leader in 19F MRI tracer development. During the course of this project we will generate critical proof of principle data for further extramural funding, peer review publications and patent applications.

**Summary of Research Completed**

Significant progress was made both in vitro and in vivo since our last report on June 30th 2011. A new imaging modality (near infrared fluorescence, NIR dye) introduced to achieve greater selectivity and sensitivity proved to be extremely good addition to our original designs. We have obtained a new in vivo imager (Li-COR Perl) which allowed us to perform NIRF in vivo imaging in a mouse breast cancer model. Further, these images were confirmed by ex vivo MRI. The work presented so far resulted in four manuscripts with the PI (Janjic) as the corresponding author: one published: O’Hanlon et al, Journal of Fluorine Chemistry 2012, 137, 27-33 one submitted (Patel et al, 2012) and two in preparation for submission at the end of July 2012 (Balducci et al, 2012 and Janjic et al, 2012). The overall achievements of Janjic group and our collaborators (Prof. Wilson Meng and Celsense Inc.) on this project are presented below in more detail. The work was also presented at two high profile international scientific meetings: American Chemical Society Fall Meeting in Denver (2011) as oral presentation (PI presenter), featured in C&E News (in article Personalizing Nanomedicine by L. K. Wolf on Aug 26th), a poster presentation (PI presenter) at the Gordon Conference: Colloidal, Macromolecular & Polyelectrolyte Solutions (Ventura, CA, 2012).

1. **Synthesis of PFPE conjugates and their formulation into novel drug carriers**
   perfluoropolyether (PFPE)-tyramide was synthesized and purified using previously reported protocol with some modifications (Scheme 1). Nanoemulsions containing PFPE-tyramide, nonionic surfactants, and a hydrocarbon oil were prepared and characterized. Microfluidization techniques yielded a stable nanoemulsion formulation with NIRF dye and celecoxib. The formulation was evaluated in vitro in a model immune cell line, Fetal Skin-derived Dendritic
Cells (FSDCs), for cellular uptake and toxicity. Cellular loading of the drug carrying nanoemulsion was evaluated by NIRF microscopy, NIRF imaging and quantified by $^{19}$F NMR. [O’Hanlon et al, JFluoChem 2012] Figure 1 illustrates typical work flow from the PFPE-tyramide construct to dual mode imaging capable drug carrier.

![Scheme 1. Synthesis of PFPE-tyramide (3) (Taken from Janjic et al, JACS 2008)](image)

**Figure 1.** Successful formulation of PFPE-tyramide into a drug carrier nanoemulsion. Figure shows the PFPE tyramide structure (top), nanodroplet proposed structure (middle), NIRF microscopy of dendritic cells carrying the nanoemulsion (left) and NIR image of the nanoemulsion *ex vivo* (right). [O’Hanlon et al, JFluoChem 2012]

2. **First COX-2 inhibiting PFPE nanotheranostic**

Combination of epidemiological, clinical and *in vitro* studies strongly suggests that COX-2 inhibition could suppress breast cancer metastasis through both direct effects on primary tumor cells and anti-inflammatory action. COX-2, an inducible enzyme involved in prostaglandin biosynthesis, is over-expressed in several epithelial malignancies including breast cancer. Elevated COX-2 expression in breast cancer tumors is associated with increased tumorigenic transformation, higher grade tumors, and decreased overall and progression-free survival (PFS) times. It was also related to increased breast cancer cell proliferation, high expression levels of p53 and human epidermal growth receptor 2 (HER2), and increased presence of metastases. Prostaglandin E2 (PGE2) is necessary for the development of immunosuppressive cells (tumor associated suppressive macrophages and myeloid-derived suppressor cells (MDSCs). These cells are critical for primary tumor immune system evasion and eventually metastasis development. Our goal is to develop a drug delivery vehicle (theranostic PFC nanoemulsion) that can provide a large dose of celecoxib at the site of inflammation yet release little or no drug systemically.

We present here a novel drug carrying perfluoropolyether (PFPE) nanoemulsion with $^{19}$F MRI and NIRF imaging capabilities. Theranostic PFPE nanoemulsion design is innovative in that: 1) It incorporates a selective COX-2 inhibitor; 2) It can serve as a multimodal biological probe for
studying the role of COX-2 in macrophage-tumor interaction; and 3) Can be imaged by two complementary molecular imaging techniques-NIRF and $^{19}$F MR imaging. The nanoemulsions reported contain the following: celecoxib, $^{19}$F MRI tracer PFPE, a NIRF dye, nonionic surfactants and water. Celecoxib is a selective COX-2 inhibitor with low aqueous solubility and high membrane permeability. The challenging task of stabilizing immiscible hydrocarbon and PFC oils was successfully achieved by using proprietary combination of nonionic surfactants. We prepared tri-phasic nanoemulsions with two immiscible oils (PFC and Miglyol), stabilized with non-ionic surfactants in water (Figure 2A). Miglyol carries the NIRF tracer and the drug.

![Figure 2.](image)

**Figure 2.** A) Proposed structure of the anti-inflammatory PFC theranostic nanoemulsion. B) DLS measurement of droplet size for the nanoemulsions with (red) and without (black) celecoxib (model COX-2 inhibitor).

Briefly, Celecoxib was first dissolved in Miglyol and PFPE oil was added followed by mixed surfactant solution and mixture stirred at r.t. A thick viscous paste was obtained to which deionized water was added and resulting mixture was stirred on ice for 15 minutes by magnetic stirring. The resulting coarse pre-emulsion was microfluidized on a Microfluidizer M110S (Microfluidics Corp., Newton, MA). The nanoemulsion was sterilized using sterile 0.22 µm cellulose filter (Millex®). Filtered nanoemulsion samples (1.5 mL) were stored at 4°C and 25°C to assess the stability. The bulk of the nanoemulsion was stored at 4°C until use. Nanoemulsions were characterized by dynamic light scattering (DLS) measurements (Zetasizer Nano, Malvern, UK) and $^{19}$F NMR (Bruker, 470 MHz). The nanoemulsion stability was followed by analyzing the particle size and PDI. The nanoemulsions samples stored at 4 and 25°C showed no change for at least 60 days with average droplet size and PDI around 150 nm and 0.15 respectively. No change in droplet size and PDI was observed upon drug incorporation (Figure 2B).

3. **Biological in vitro testing of celecoxib carrying PFPE theranostic nanoemulsion**

*In vitro* toxicity studies were conducted using Celltiter-Glo® Luminescence Cell Viability Assay to ensure that the nanoemulsion is nontoxic. Mouse macrophages (RAW 264.7, ATCC) were chosen as the model inflammatory cells. As shown in figure 3A, no significant effect on cell viability was detected after 24 h exposure to the nanoemulsion. The values are reported as percent control (unexposed cells).
Figure 3. A) Cell viability test in mouse macrophages upon 24 h exposure to PFC nanoemulsions with/without celecoxib. Viability was assessed by Cell Titer Glo® luminescence assay (Promega). B) Production of PGE2 in macrophages assessed after LPS treatment. LPS treatment was performed post labeling. Each data point represents the average and error bars are the standard error of the mean (n=9). [Patels et al, 2012 Submitted]

To investigate the \textit{in vitro} therapeutic efficacy of the drug carrier, effect of nanoemulsions on PGE2 production by macrophages was assessed. Inflammatory cells in the tumor environment express elevated levels of COX-2. COX-2 is involved in the biosynthesis of PGE2, which in turn supports tumor progression. Because PGE2 is released into the cell culture medium, macrophages were first exposed to the nanoemulsions for 24 h, nanoemulsion was removed and then activated using LPS for 3 h. LPS activated cells showed more than two fold increase in PGE2 as compared to untreated. COX-2 inhibition was evident from the reduced production of PGE2 by cells labeled with celecoxib and dye loaded nanoemulsion as compared to nanoemulsions without drug (Figure 3B). Dual mode imaging capability is illustrated in figure 4 where the same emulsion is detected in labeled macrophages by both $^{19}$F NMR and NIRF imaging. Cellular uptake of the nanoemulsion was also confirmed by confocal NIRF microscopy (Figure 5). [Patels et al, 2012 Submitted]

Figure 4. (A) $^{19}$F NMR of cells labeled with PFPE nanoemulsion. 0.02% v/v aqueous TFA set at -76 ppm was used as reference for $^{19}$F NMR. (B) NIRF image (at 800 nm) of cells labeled with PFPE nanoemulsion in NMR tube. [Patels et al, 2012 Submitted]
Figure 5. Confocal imaging of macrophages labeled with COX-2 inhibitor carrying PFPE nanoemulsion (Images by JA Pollock, 2012). Images strongly support the notion that PFPE nanoemulsions are located in the cell cytoplasm. [Patels et al, 2012 Submitted]

4. Preliminary Feasibility Studies for $^{19}$F MRI and NIRF Imaging of Inflammation in a Mouse Breast Tumor Model

The following experiments were conducted to test the feasibility of drug delivery to breast tumor inflammation specifically. For that purpose we first used a NIRF/$^{19}$F MR nanoemulsion that has high capacity of labeling inflammatory cells upon i.v. injection. This nanoemulsion does not incorporate drugs but can help us determine the level of inflammation in a breast cancer mouse model by two imaging techniques: $^{19}$F MRI and optical NIRF imaging. Live in vivo NIRF imaging of accumulating macrophages to tumors was performed using the prototype NIRF/$^{19}$F MR reagent V-Sense 580H Dual Mode NIR provided by Celsense Inc (Pittsburgh, PA). The reagent was injected in the tail vein and mice imaged on the Li-COR Pearl Imager at 800 nm emission detection, figure 6. These are first live images ever reported to the best of our knowledge of tumor associated inflammation in a breast cancer mouse model. (Mouse experiments and imaging performed by Yi Wen, Meng group, Duquesne Univ. 2012)
Conclusions on the reported progress:

1. Feasibility of dual NIRF and $^{19}$F MRI imaging in a mouse breast cancer model was demonstrated by \textit{in vivo} NIRF optical imaging and \textit{ex vivo} $^{19}$F MR analysis of excised tumor tissues.

2. Two perfluorocarbon nanoemulsion formulations were developed and one displayed dramatic effects on COX-2 activity in macrophages.

3. The work summarized above resulted in 4 publications at different stages and two major international meeting presentations.
Research Project 2: Project Title and Purpose

Promoting Health and Health Care Access in the African Refugee and Immigrant Community: A Participatory Action Research Study - The purpose of this Participatory Action Research project is to understand specific culturally shared knowledge about health and to develop strategies to promote health and health care access in the African immigrant and refugee community. The overall goal is to engage the African immigrant and refugee community in identifying, planning, prioritizing and evaluating strategies to promote health from the unique cultural view and to empower people to create their own destiny regarding the reduction of health disparities in this community.

Anticipated Duration of Project

1/1/2011 - 12/31/2013

Project Overview

The broad research objective for this project is to gain an understanding of specific cultural beliefs, values and strategies to promote health and health care access in the African immigrant and refugee community. The specific aims of the research are to: 1) explore the health promoting needs of African immigrants and refugees; 2) describe the health care access needs of African immigrants and refugees; 3) understand the culturally congruent process of developing strategies within the specific immigrant and refugee community to address the health promoting and health access needs of the community; and 4) compare the perceptions of self-reported health status for African immigrants and refugees at the beginning and end of the study.

Method and design: This Participatory Action Research (PAR) project will utilize a mixed methods approach, including focused ethnography and the Short Form Health Survey Instrument (SF-12), to gather data at the beginning and end of the study from the informants. Four common characteristics of PAR are: 1) uncovering solutions to health problems; 2) collaboration between researchers and the community; 3) implementation of change during the process; 4) and development of a theory. The design includes a cycle, which includes the plan, action, observation, reflection, and plan or revised plan, followed by acting, reflecting, and evaluating.

Informants and setting: Informants for this study will be recruited from the Pittsburgh and Allegheny County area and include any adult members (>18-90 years of age) from the African immigrant and refugee community who are willing to participate in the study. The researchers will seek out approximately 50 to 60 adults for the core and focus groups for this study. In addition, the groups will include a researcher, nursing faculty, and the Executive Director of Acculturation for Justice, Access and Peace Outreach (AJAPO). Informants will be purposefully sought via word of mouth and snowball method by AJAPO from cultures (Somali, Burundi, Sudanese, Liberian, Zambian) representing the African immigrant and refugee population in this city.

Instruments: A researcher-generated, semi-structured interview guide and demographic form will be used to understand specific cultural knowledge and strategies to promote health and health
care access in the African immigrant and refugee community. In addition, the SF-12 will be used to measure (pre and post-intervention) eight domains of health: physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, social functioning, role limitations due to emotional problems, and mental health.

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Yinka Aganga-Williams, PhD – employed by Acculturation for Justice, Access and Peace Outreach

**Expected Research Outcomes and Benefits**

The primary expected outcomes and benefit are that the community can immediately use the findings from this project to promote health and health care access for this population. Through the process of Participatory Action Research, members of the community are part of the research process in identifying problems and solutions and can control their own destiny regarding health and health care access based on their unique cultural values. It is significant that care and health care needs be articulated and understood and treated in the cultural context of the people being served. The immigrant population in this community experiences health disparities due to their immigrant or refugee status. Research with outcomes that result in immediate benefit to the community is imperative.

The African immigrant and refugee community, which utilizes the services of AJAPO, is plagued with problems related to economic issues, social concerns, violence, political impact, and other health-related issues. Nurses, collaborative health care professionals, and community members can and will work together to form partnerships that promote healthy communities. The goal of empowering individuals, families, and communities to create their own destiny regarding their health and health care access is the ultimate benefit of this research. The expected outcomes and benefits of this project include a community-academic partnership with the continued goal of promoting quality health care to this underserved community.

**Summary of Research Completed**

The study has progressed to the near completion of phase one with the exception of one focus group with members of the Liberian community. Participatory action research is the method being used for this study and places importance on collaboration with researcher and informants as co-researchers during the entire process. The underlying belief is that the shared process of
participatory action research will empower community participants in the process and improve health outcomes. Phase one of the study employed a qualitative approach in the form of focused ethnography along with a quantitative exploratory self reported health status tool (SF-12) for descriptive data. A focused ethnography was used to elicit data from the unique cultural perspective of the informants about their health needs and health access. The study started with focused ethnography (focus group) for the core group, which included representative leaders of the five cultures (Zambian, Somali, Burundi, Sudanese, Liberian) to determine the plan for assessment in the community. A total of four focus groups (July 9, August 13, September 24 and October 29) were held with the core group and included a total of 12 informants (3 female and 9 male). Each member of the core group completed the SF-12 and participated in a semi-structured interview in a group setting about their role as co-researchers and the identified and perceived health care needs of their perspective community. The data is being analyzed separately as a core group and in the context of and along with the overall data from the perspective communities for thematic commonalities for the interventions in phase two of the study. Core group members agreed to encourage members of their specific community to participate in the research study.

After completion of the focused ethnography with the core group the study continued with members of the larger community (Zambian, Somali, Burundi, Sudanese, Liberian). A focused ethnography was also used with members of each specific community to understand the culturally specific health promoting needs and health access. The consent procedure, informed consent (obtained), demographic forms and the project time-line and the next steps of the project were discussed in detail. A total of four focus groups were held with members of the representative cultures (Zambian, Somali, Burundi, Sudanese) and included a total of 20 informants (10 female and 10 male). Please see table 1.

The researchers have not yet interviewed members of the Liberian community due to scheduling difficulties. Each member of the culturally specific focus group completed the SF-12 and participated in a semi-structured interview in a group setting about their identified and perceived health care needs and health access of their perspective community. Informants were asked about solutions to identified problems and concerns related to health and health care access as well as the role of health care professionals in promoting health.

Data analysis has been occurring concurrently with data collection. The data for each community is being analyzed separately and with the core group and in the context of the overall data from the perspective of all the communities for thematic commonalities for the intervention in phase two of the study. The Four Phases of qualitative data analysis are being used for this study. The phases include: 1) collecting and documenting raw data, 2) identification of descriptors and categories according to the domains of inquiry and research questions, 3) identifying patterns and contextual analysis, discover saturation of ideas and recurrent patterns, and 4) identification of themes and theoretical formulations and recommendations. Nvivo9 qualitative data manager is being used to manage the data for analysis. Initial data analysis from all the focus groups (core group and specific communities) suggest the following categories from phase two of data analysis:

1. Poverty as a barrier to health
2. Cultural imposition in the health care setting
3. Health care is expensive
4. Much concern for women’s health in the US
5. Little concern for men’s health in the US
6. Domestic violence
7. Language barrier
8. Difficulties with accessing affordable health care
9. Promoting health through the faith community
10. Navigating the health care system
11. Lack of cultural support in the community
12. Using the culture and elders to support health
13. Forming partnerships
14. Lack of cultural understanding by healthcare professionals
15. Misunderstandings about the US health care system

Acculturation for Justice, Access and Peace (AJAPO) provided interpreters for the focus groups for the core group and four communities in the following languages: English, French, Arabic and Swahili. The team continued to elicit support from the community members via phone calls and emails. The intention was to maintain interaction from each specific and unique community about health needs and health care access through participation in the ethnography and self-perceived health status evaluation. Food and refreshment were provided at all focus group sessions and facilitated by the AJAPO staff.

Table 1

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