Duquesne University

Annual Progress Report: 2010 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

Formula Grant Overview

The Duquesne University received $116,091 in formula funds for the grant award period January 1, 2011 through December 31, 2014. 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.

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 $(CF_2)_n$ or perfluoropolyethers (CF_2CF_2O), 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}F NMR will be used to measure ^{19}F content, evaluate fluorocarbon-hydrocarbon interactions and assess the potential "imageability" of each vehicle by ^{19}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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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 ^{19}F 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

1. *Colloidally and Photostable Triphasic Nanoemulsions – improved formulations for maximized imaging and drug delivery capacity*

Typical nanoemulsions are composed of two phases, oil and water, while triphasic nanoemulsions have three phases (PFC, HC and water). During the current reporting period, 7/1/2013-12/31/2013, our data indicated that changes in composition can maintain small droplet size (100-200nm) and small polydispersity index (PDI) (<0.2). This is advantageous because it would allow adaptations to imaging and drug carrying without significant changes needed in the formulation process. Presence of drug does not affect size distribution (Figure 1A). We observed no change in size of droplets as measured by dynamic light scattering (DLS) when our recently developed quaterrylene-incorporated PFC nanoemulsions are dispersed in water, serum free, or 10% and 20% serum containing media stored at 37 °C for 72 h, attesting to high colloidal stability. Upon storage for > 180 days no change in size was observed. Another example was stable up to 2 weeks under same conditions (Figure 1B). In our preliminary study, we showed that triphasic PFC nanoemulsions incorporated with a quaterrylene dye showed negligible NIR fluorescence signal change when exposed to ambient light for 2 days (Figure 1C), whereas the same emulsions carrying a commonly used commercially available NIR dye (DiR) were completely photobleached. In addition, quaterrylene dyes (developed by Dr. M. Bai at University of Pittsburgh) showed greatly improved fluorescence when dissolved in Miglyol or nanoemulsions, as compared to aqueous media (DMEM), Figure 1D. Therefore, we chose quaterrylene dye loaded PFC nanoemulsions as a platform to engineer the proposed new targeted and anticancer drug loaded bimodal nanoemulsions. The critical requirement for targeted drug

delivery is that the vehicle (drug-free nanoemulsion) is not causing changes in cell viability, and a pharmacological effect only comes from the delivered drug. Figure 1E shows no toxicity in cells upon 24h exposure to drug-free nanoemulsion. In Figure 1F fluorescence microscopy clearly indicates their intracellular location. Based on these preliminary data, generated in the last C.U.R.E. funding period, we published a paper in high impact factor journal and submitted an NIR R21 proposal to NCI in May 2014 titled: Multifunctional Nanoemulsion Platform with High Photo and Colloidal Stability (PIs: J.M.Janjic and M. Bai (University of Pittsburgh).

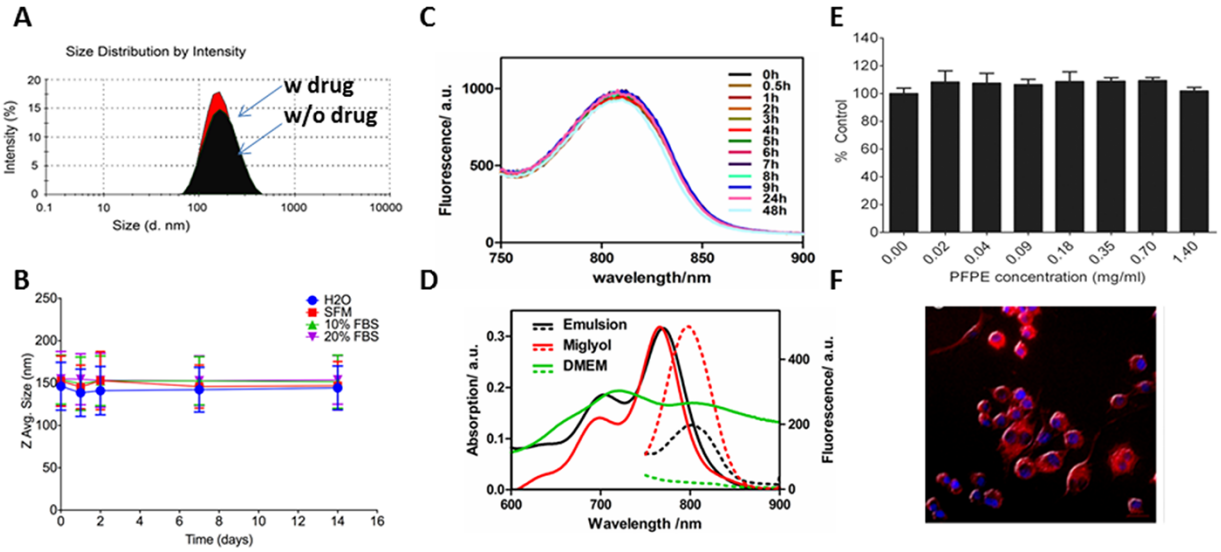

Figure 1. NIR labeled triphasic PFC nanoemulsion characterization. A) size and polydispersity with/without drug; B) stable in serum containing culture media for 2 weeks; C) photostable upon prolonged light exposure; D) fluorescence spectra of quaterrylene NIR dye (QR-4Py) in emulsion, oil and media; E) no change in cell viability upon 24h exposure to increasing drug-free nanoemulsion concentrations; F) nanoemulsion droplets (red) in cells.

2. <u>Successful introduction of NIR labeled and drug loaded PFC nanoemulsions into hydrogels</u>

Local delivery is advantageous over intravenously injected nanoemulsions by decreasing systemic exposure and potential toxicity. In earlier reporting period we discussed PFC based macrogels, formulated by cross-linking PFPEs with polyamines in situ in an aqueous polymer solution. In this reporting period (7/1/2013-12/31-2013) our newest work on macrogels that incorporate drug loaded and NIR labeled PFC nanoemulsions. These formulations are termed emulgels. By definition are formulations where nanoemulsions are incorporated into polymer based hydrogels. Nanoemulsions, prepared by microfluidization, are incorporated into thermoresponsive polymer based hydrogel matrix that upon incubation at select temperatures results in an emlgel. The resultant emulgels are monitored for stability and imaging properties

over time. Stability is assessed by dynamic light scattering droplet size and zeta potential measurements, rheological measurements and pH. UV/VIS measurements are used to measure drug loading and NIR imaging on LiCOR Odyssey to assess NIR labeling and optical properties of the gels. Table 1 lists selected emulgels and Figure 2 summarizes key results from this ongoing study. The results from this work were presented at McGowan Research Institute for Regenerative Medicine Scientific Retreat in March 2014.

Table 1. Select emulgels prepared with PFC nanoemulsions

Gels	PFC emulsion	Surfactant	Dye
D	PFPE oxide	Pluronic®	No
E	PCE	Pluronic®	Yes
F	PFC free	Pluronic®	Yes
G	No	Pluronic®	No

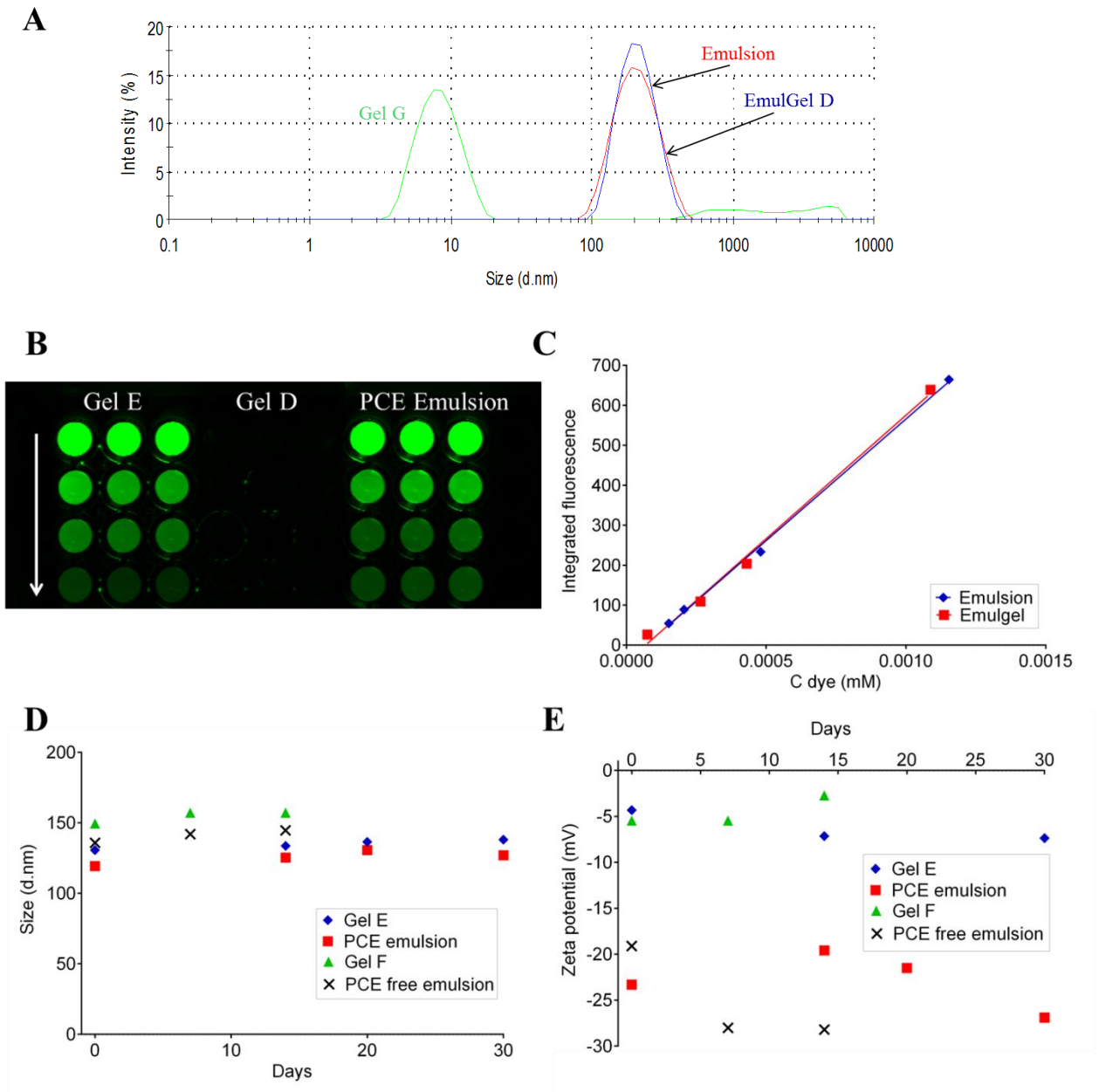

Figure 2. Characterization of the emulgels described in Table 1. A) Particle size comparison for gels D, G and the corresponding PFPE oxide emulsion (Table 1) at 0 time; B) Li-COR Odyssey imaging for different concentrations of dye in gels D, E and PCE emulsion with dye at 800 nm; C) linearity curves of dye in gel E and the corresponding PCE emulsion; D) particle size stability for gels E and F and the corresponding emulsions at 25 °C and 4 °C respectively and E) zeta potential stability for gels E and F and the corresponding emulsions at 25 °C and 4 °C respectively

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 - 12/31/2014

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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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 majority of phase one of this study has been completed during this reporting period. Phase one will inform and provide recommendations for interventions in Phase two of the study. The major focus of the study for this year has been on data collection and data analysis of the focused

ethnography to the thematic level. The study used a community-engaged process, which included a focused ethnographic method. The method called for focus group interviews with members of five specific cultural groups (Somali, Burundi, Sudanese, Liberian, Zambian) representing the African immigrant and refugee population in the City. Members of all cultural groups except the Liberian community had previously been interviewed. Repeated efforts were made by the researchers to secure a time and place for the group interviews with the Liberian community since the last report. In October of 2013, three members of the Liberian community were interviewed and their data was added to the raw data for analysis.

The original plan for data collection and analysis changed from the beginning of the study. Due to the transient nature of many of the informants it became evident that the researchers would not be able to identify and re-interview and re-administer the SF-12 to the original informants. Due to the varying numbers of informants from each community, the data was analyzed together. The decision to analyze the data together has been supported and confirmed in the analysis of the data. With the inclusion of data from the Liberian community the data showed similarities in categories, patterns and themes among all of the five cultural groups represented in the study. This process of data analysis is consistent with the ethnographic method and Leininger's phase of Qualitative data analysis.

All the data from the study was analyzed concurrently with data collection as well as after the most recent interviews. The data for each community were analyzed with the core group and in the context of the overall data from the perspective of all the communities for categories, patterns and themes inherent in the data. Three phases of qualitative data analysis were completed previously: 1) collecting and documenting raw data, 2) identifying descriptors and categories according to the domains of inquiry and research questions, 3) identifying patterns and contextual analysis and discovering saturation of ideas and recurrent patterns. During this reporting period, we completed the fourth phase of data analysis, which consisted of identification of themes and theoretical formulations and recommendations.

Nvivo10 qualitative data manager was used to manage the data for analysis. Portions of the demographic data and analysis of the SF-12 have been completed but are not yet ready to report in the context of the qualitative ethnographic findings.

After continued analysis of the data during this reporting period there were some changes in the number and description of categories, patterns and final identification of themes for this study. Originally there were 21 categories identified. After continued analysis the following categories were identified and re-written in phase two of data analysis:

1. Culturally defined illness
2. Difficulties with assessing affordable health care
3. Family
4. Forming partnerships
5. Health
6. Health care is expensive
7. Lack of cultural pride and support in the community
8. Lack of cultural understanding be health care professionals and institutions
9. Language barrier

10. Little concern for men's health in the US
11. Long road to the US
12. Misunderstanding about the US health system
13. Navigating the health care system
14. Personal care
15. Poverty as a barrier to health
16. Promoting health through the faith community
17. Specific cultural care
18. Too much concern for women's health in the US
19. Using Elders to support health
20. Violence

In the prior report eight patterns were identified in phase three of data analysis. After continued analysis two of the eight patterns were changed to reflect the deeper level of analysis. The changed patterns were:

1. Pattern of poverty and health care expense as a barrier to health care
2. Pattern of needing and wanting education in native language if possible and better health care access

The final phase of data analysis for the focused ethnography included the identification of themes. The following themes for this study are:

1. The Somali, Burundi, Sudanese, Liberian and Zambian refugee communities share a common journey with various stops and lengths of stays from their country of origin to the United States
2. Poverty, inability to pay for health care serves as a major barrier to heath care access for African refugees and immigrants
3. Language, social isolation and mistrust of health care professionals and institutions restrict the use of health care services for African refugees and immigrants
4. Community and family violence is a major health care issue due to lack of community and Elder support and misunderstandings of the host culture

Demographic data for this study is continuing to be analyzed in the context of the qualitative and quantitative data. Report of demographic data of the informants can be found below.

Demographics	
Ethnicity	Number
Liberian	4
Sudanese	4
Burundi	8
Zambian	7
Somali	7
African	2
Total	32

Gender	Number
Male	18
Female	14

Own/Rent Home	Number
Own	1
Rent	30
No Response	1

Marital Status	Number
Never Married	8
Married	18
Living with Partner	1
Separated	3
Divorced	1
No Response	1

Highest Level of Education	Number
Grade School or Less	15
High School Graduate	7
Some College	4
College Graduate	2
Graduate Degree	1
No Response	3

Health Care Provider	Number
Yes	19
No	11
No Response	2

English as Primary Language	Number
Yes	12
No	2
No Response	18

Income	
Dollar Amount	Number
<$10,000	10
$10,000 - $19,000	9
$20,000 - $29,000	7
$40,000 or More	1
No response	4

U.S. Citizenship	
Yes	17
No	7
Refugee	1
Permanent Resident	6
No Response	1

Health Insurance	Number
Yes	20
No	11
No Response	1

Number of People in Household	
Average	4.966

Years of Education	
Average	7.766