CROSSROADS

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West Long Branch, NJ

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TABLE OF CONTENTS

ABSTRACTS

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THESES

EDITOR'S NOTE

Crossroads is an interdisciplinary, undergraduate research journal published by the Honors School at Monmouth University. The contributors are Junior and Senior Honors thesis students whose work has been chosen by the Honors Council as representing the most original, thoroughly researched, and effectively argued theses in their fields.

Crossroads is made possible through the support of Monmouth University and the generosity of our benefactor Ms. Jane Freed, class of 1981. The articles in this volume include works in the fields of: Biology, Chemistry, English, Music, & Psychology.

Deep gratitude must also be given to the Chief Advisors and Second Readers. It is through their inspiration and support that our Honors School students succeed. Without their mentorship, the students would be missing out on a key component of their experience in the Honors School.

Additionally, we must thank Ms. Erin Hawk and Ms. Reenie Menditto for their help in advising and supporting all thesis students. Without their care and attention, *Crossroads* would not be what it is today.

Lastly, we must thank Professors Neil Graves and Kenneth Mitchell; the Honors Thesis Advisors.

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DISCOVERING THE ELEMENTS OF THE CLASSICAL CLARINET CONCERTO

EMILY STEEBER

ABSTRACT

The Classical era in music was a time of great development, instrumental innovation, and structural maturation; many composers used the latter half of the eighteenth century to compose for new instruments and change the world of music forever. This creative thesis project explored the various elements implemented in the first two movements of two similar Classical clarinet concertos: Wolfgang Amadeus Mozart's Clarinet Concerto in A Major, K. 622 and Karl Stamitz's Clarinet Concerto No. 3 in B-Flat Major. Through harmonic and melodic analyses and studying the historical and cultural context in which these concertos were created, I was able to create my own movement of a Classical clarinet concerto that synthesized the elements observed throughout the Mozart concerto and the Stamitz concerto.

A video of the lecture-recital where this creative thesis was presented can be viewed on YouTube.

ACKNOWLEDGEMENTS

Many people supported me throughout my journey towards composing my first works for clarinet. Without these people, this creative thesis and the presentation of my senior lecture-recital would have been impossible.

First, I need to express gratitude to my chief advisor and second reader, Professor Tony Tafrow and Professor Michael Gillette. Professor Tafrow helped me through each step of my creative thesis, down to the minute before my senior lecture-recital presentation. Professor Gillette's unending knowledge of music history was especially helpful early on in the thesis process. I cannot thank these two outstanding professors enough.

Dr. Dooley and Dr. Graves helped me to stay on track and shape my creative thesis topic and guided me through the thesis process.

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A huge thank you must go out to the faculty, staff, and students of the Department of Music and Theatre Arts, especially Professor Laura DuBois for her endless hours of rehearsal and performance accompaniment; Professor George Wurzbach for assisting with the sound for my lecture-recital; Henry Siebecker for providing the stage with lighting; Joe Callandrillo for turning pages; and Dr. Gloria Rotella for being the most incredible and supportive academic advisor.

Additionally, I must extend my gratitude to everyone who has encouraged my love for music since the very beginning, especially my parents, Desireé and Eric; my siblings, Amanda, Brian, Erica, and Jillian; my boyfriend, Brandon Rossi; and my dog, Mozart. I appreciate all of your constant love, support, and inspiration.

G-QUADRUPLEXES AS TARGETS FOR ANTICANCER DRUGS

CORTNEY CAVANAUGH

Abstract

The American Cancer Society estimates that in the year 2013, there will be approximately 1,660,290 Americans diagnosed with some form of cancer, besides basal and squamous cell skin cancers.[1] Consequently, a great deal of research is focused on curing the disease. The purpose of this research is focused on describing the use of G-quadruplexes as targets for anticancer drugs. In addition to a review of the literature, the synthesis and biological studies of novel potential anticancer drugs will be discussed.

Many ligands have been synthesized for the stabilization of G-quadruplexes in recent years and these ligands consist of several common characteristics. Based on these characteristics, several naphthalenediimide (NDI) derivatives have been proposed as potential G-quadruplex stabilizing ligands. Five novel ligands were successfully synthesized. DNA melting experiments were carried out on compounds **4** and **6** to test their ability to target G-quadruplexes. Both ligands demonstrated minor success in stabilizing these structures with ΔT_m values of 3.5 °C for compound **4** and 5.9 °C for compound **6**.

ACKNOWLEDGEMENTS

First I would like to thank Dr. Lamberto for his support as a professor, academic advisor, research advisor, mentor, and chief advisor for my thesis. The opportunities that have been made available to me, as a member of his lab, have greatly influenced my success as a student at Monmouth University. It is because of his courses that I have developed a passion for organic chemistry and will be perusing my interests in graduate school. I cannot thank him enough for all that he has done for me.

I am also very appreciative for all that Dr. Tongesayi has done for me as the second

reader for my thesis. He was always available to give me guidance and support. Beyond his contributions to my education as a professor, Dr. Tongesayi has helped me greatly during the graduate school application process. Thank you for everything Dr. T.!

A significant portion of my research was conducted during the Summer Research Program. For that, I would like to thank the Monmouth University School of Science for its support and Dr. Lamberto for allowing me to work in his lab.

Finally, I would like to thank the Honors School for giving me the opportunity to write my thesis. Thank you Dean Dooley, Reenie Menditto, and Erin Hawk for all that you do.

Introduction

G-quadruplexes are structures that are present in the telomeres of chromosomes. They have proven to be viable targets in the treatment of cancer. A significant amount of research has been directed at determining the physical conditions that promote their existence and developing drugs with the ability to stabilize them. To fully understand their role in the ongoing fight against cancer, it was necessary to obtain a thorough description of the general structure, discovery, and use of G-quadruplexes. Published ligands were evaluated for their ability to promote and stabilize the G-quadruplex structure. A literature review supported original research, which included the synthesis of several novel ligands and the testing of their ability to stabilize G-quadruplexes.

Background

A widespread dilemma plaguing many cancer treatment methods, including radiation and chemotherapy, is the lack of specificity in regard to the target cells. In addition to cancer cells, healthy cells are also at risk of being destroyed by the treatment processes. However, the stabilization of G-quadruplexes will only destructively interfere with the replication of cells that are under the influence of the enzyme telomerase.

Telomeres are G-rich sequences located at the ends of human chromosomes that prevent the loss of critical coding sequences after a round of DNA replication.[2] The telomere of a chromosome is initially 10 to 20 kilo base pairs in length. Between 25 and 200 base pairs are cleaved during each round of cell division. Eventually, the telomeres reach a critical length (4 to 6 kilo base pairs) at which the cell is no longer able to replicate.[3] This is known as the Hayflick limit and when it is reached, apoptosis of the cell is triggered. This mechanism is often problematic in the case of cancer cells, where unregulated replication is a consequence of the enzyme telomerase. The action of telomerase prevents the depletion of the telomeres and helps to elongate them. The enzyme is produced minimally in somatic cells but is continuously produced in many cancer cells. It has been determined that telomerase is expressed in more than 85% of tumors.[4] Researchers have observed that G-quadruplex structures are found *in vivo* and *in vitro*. Their presence in telomeric DNA can interfere with telomerase activity and prevent the uncontrolled elongation by telomerase that is observed in cancer cells.[3]

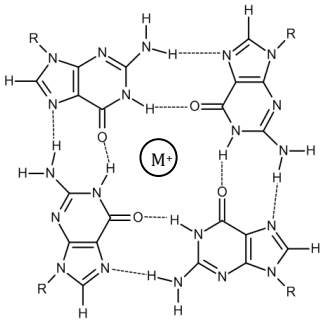


FIGURE 1. A planar G-quartet constructed of four guanine residues connected through Hoogsteen bonds.[7]

In 1910, DNA solutions, rich in the nucleobase guanine, were observed to form gelatinous aggregates. Further analysis of this discovery remained on hiatus until 1962 when a research team, led by Martin Gellert, suggested that the gels arrange in a cyclic manner through the stacking of planar, quinine regions.[6] Telomeres consist of numerous guanine rich sequences of DNA, generally TTAGGG. These guanine-rich regions form G-quartets, which are the cyclic, planar arrangements of four guanine bases, as proposed by Gellert et al. (FIGURE 1). The four guanines are held together through a series of eight hydrogen bonds, known as Hoogsteen bonds, rather than the Watson-Crick bonding observed in most regions of DNA. The overall quartet is stabilized by the presence of a monovalent cation, usually K^+, Na^+, or Cs^+. Highly stable G-quadruplexes are formed when a number of G-quartets stack directly on top of one another (FIGURE 2).[7] The signature stacking is the result of π-π non-bonded attractive interaction, which increases the stability of the G-quadruplex.[8] The presence of G-quadruplexes within the telomeric DNA sequence negatively interferes with the enzymatic activity of telomerase. As a result, the normal life progression of the cell proceeds, ultimately resulting in apoptosis.

Specificity of the ligands, or the G-quadruplex promoting and stabilizing molecules, is crucial in the synthesis of anticancer drugs. The vast majority of somatic cells are unaffected by the presence of telomerase. Despite this fact, some researchers fear that the ligands may exhibit an adverse effect on normal cells, in addition to tumor cells. However, studies conducted on previously synthesized ligands have shown excellent selectivity for cancer cells over normal cells for unknown reasons.[5] Consequently, G-quadruplexes can be exploited to inhibit the aggressive

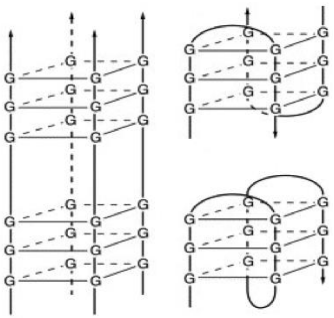


FIGURE 2. Series of G-quartets stacked into the formation of G-quadruplexes where (G) represents the guanine residues.[7]

replication observed in cancer cells, due to the activity of telomerase. This is accomplished by stabilizing the structures through the binding of a ligand. Future research can be focused on determining whether uniquely high levels of telomerase, present in the cancer cells, account for the selectivity of the ligands.

Significant interest has been directed towards this field of research and as a result, a variety of successful ligands have been identified to date including tetra-(N-methyl-2-pyridyl)porphine (TMPyP2), tetra-(N-methyl-4-pyridyl)porphine (TMPyP4), 3,3'-diethyloxyadicarbocyanine (DODC), and ethidium bromide (EtBr) (FIGURE 3).[7] An important component of

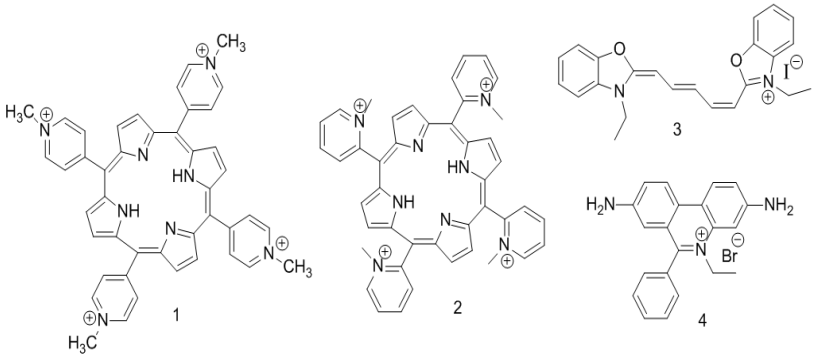


FIGURE 3. Several ligands have been synthesized and shown to exhibit apparent G-quadruplex stabilizing capabilities. These ligands include TMPyP4 (1), TMPyP2 (2), DODC (3), and EtBr (4).[7]

synthesizing the ligands is the development of a core structure from which a variety of derivatives can be developed. Once a basic skeleton is developed, it is possible to increase the selectivity and binding strength of the ligand by varying the functional groups on the molecule. The ligands become nested within the grooves of the G-quadruplex and stabilize the structure. To promote this positioning, it is favorable for at least two side chains to be directed toward the quadruplex grooves with a tertiary amine positioned at the termini of the side chains.[9] At the physiological pH, the amines should be protonated. In order to promote the stacking of G-tetrads, the structure of the ligand should include a series of planar, aromatic rings in the central region of the molecule. Highly successful ligands are often derivatives of bicyclic heteroaromatic

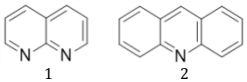


FIGURE 4. The efficiency of 1,8-naphthyridine (1) and acridine (2) based compounds, as potential G-quadruplex stabilizing ligands, has been studied.

compounds such as 1, 8-naphthyridine. However, the use of acridine-based ligands is not highly recommended due to the potential risks of instability during the final steps of the preparation process and storage (FIGURE 4). Such derivatives exhibit instability due to the result of the hydrolysis of the amide bonds on the acridine ring.[10] Due to the presence of loops, grooves, and the negatively charged sugar-phosphate backbone of the G-quadruplex DNA, it is also suggested that the substituents of the ligands are positively charged to promote interaction with these regions.[11] Through the discovery and analysis of core ligand structures, it is possible to make modifications, which can promote stronger and more specific binding.

The successful binding of a ligand to a G-quadruplex can be analyzed through multiple methods. Common methods include fluorescence intercalator displacement (FID) assays, DNA melting experiments, and ultra violet thermal difference spectra (UVTDS). In an FID assay, the quadruplex structures are mixed with thiazole orange dye and are titrated with the synthesized ligands. The thiazole orange dye indicator is displaced with the addition of the ligand, which results in a decrease in fluorescence. A plot of the ligand concentration against the percent displacement of the dye demonstrates the binding strength and selectivity of the ligand. Greater quantities of displacement indicate a high affinity between G-quadruplexes and the ligand under analysis.[12] The ability of a ligand to promote and stabilize the formation of G-quadruplexes is also studied through DNA melting studies, which are based on melting temperatures. The analysis measures the shift in melting temperature of the G-quadruplex DNA, or the temperature at which half of structures denature. The studies are carried out in a temperature-controlled UV-VIS and the absorbance is plotted against the temperature. Fluorescence resonance energy transfer (FRET) can be used in addition to the melting studies to measure the shift in relation to the ligand concentration.[11] This technique also provides insight into the conformations in which the structures exist. Small regions of bases, which fold into a G-quadruplex, are labeled with donor and acceptor fluorescent tags. As the structure folds, the energy of the donor fluorophore is transferred to the acceptor. The efficiency of the transfer indicates how tightly the G-quadruplex is folded and the conformation in which the structure exists when in the presence of a ligand. In UVTDS, the absorption spectra between the folded and unfolded G-quadruplex are compared. Obtaining UV spectra over a range of temperatures will indicate when the stabilized G-quadruplexes unfold and provide thermodynamic data including the change in enthalpy,

melting temperature, and the change in Gibbs free energy. This data allows the stabilizing ability of several ligands to be compared. Higher melting temperatures indicate greater stability of the G-quadruplex and a greater effectiveness of the ligand.[5] Being aware of the effectiveness of ligands in binding the G-quadruplex structure allows researchers to determine whether the compound is a viable anticancer drug or if more work is required to increase its potency.

Three structures proposed and tested by Dhamodharan et al. were

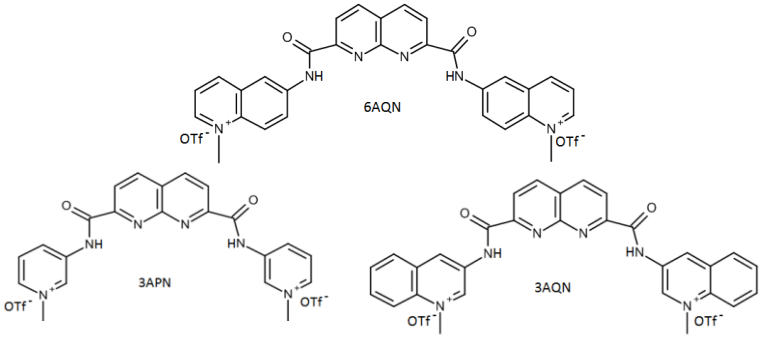


FIGURE 5. Ligands proposed and synthesized by Dhamodharan et al.. They have shown quadruplex stabilizing abilities and are referred to as 3AQN, 3APN, and 6AQN.[12]

particularly interesting for quadruplex stabilization. The ligands, called 6AQN, 3AQN, and 3APN, were very successful at promoting and stabilizing the G-quadruplex structure (FIGURE 5). Dhamodharan et al. selected structures with multiple aromatic regions and two sites with positive charges. It was predicted that the combination of these features would result in successful stabilization. The stabilizing effects of these ligands were tested using DNA melting studies. It was reported that the change in the telomeric DNA melting temperature for 3AQN was 21.0 °C. This change indicates that the ligand has very strong quadruplex stabilizing capabilities. The change in melting temperature for 6AQN and 3APN were 15.1 and 7.7 °C, respectively. It was likely that the smaller change in melting temperature for compound 3APN was the result of shorter side chains, which are inefficient in forming a strong interaction with the G-quadruplex.[12] The central, naphthyridine core of these structures is credited with stabilizing the quadruplex. Based on the

information accumulated in the review of the literature, novel G-quadruplex stabilizing ligands were proposed, synthesized, and tested.

Objectives

Several, potential G-quadruplex stabilizing ligands have been synthesized and tested. These innovative NDI derivatives were developed based on common characteristics of successful ligands found in the literature. Using microwave irradiation, five ligands were efficiently synthesized in good yields. The effectiveness of compounds **4** and **6**, as G-quadruplex stabilizing ligands, was studied using DNA melting experiments.

Experimental- Materials and Methods

Ligand Design

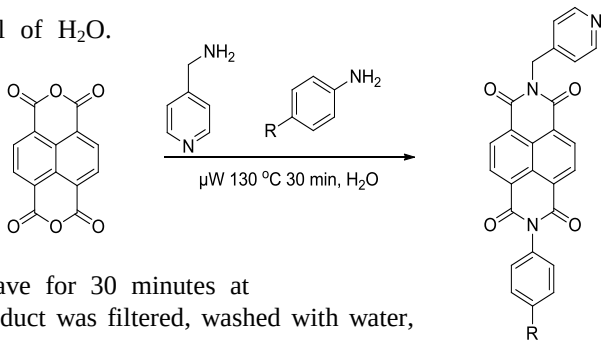
Five NDI derivatives were synthesized for the purpose of stabilizing G-quadruplexes. The basic NDI structure was selected because it provided a central planar core constructed of four rings. This planar region was expected to aid in the efficient stacking of G-tetrads. Two side chains were added to the core structure. The addition of substituted amines to the original structure added more aromatic rings to the molecules. Alkylation of the asymmetric molecules allowed for variations in the core structure. More aromatic rings or less bulky alkyl groups were added to the structure using alkyl halides. Varying the R-groups of the structures made it possible to identify potential functional groups that aid in ligand-quadruplex interactions. The addition of the alkyl group to the nitrogen of the substituted pyridine ring resulted in a positive charge. This was expected to interact with the negatively charged sugar-phosphate backbone. The successful synthesis of these asymmetric NDI ligands opened a new class of ligands that allowed for the synthesis of a large variety of derivatives.

Synthesis of asymmetric NDI 1-3

Based on the procedure found in Pengo et al., asymmetric NDI **1**-**3** were synthesized in excellent yields by reacting 1,4,5,8-naphthalenetetracarboxylic dianhydride with equimolar amounts of 4-(Aminomethyl)pyridine and one of three substituted amines (p-Toluidine, aniline, and 4-Aminophenol for compounds **1**, **2**, and **3** respectively) (SCHEME 1). The three reagents were added to a microwave tube and

SCHEME 1. General synthetic method for asymmetric NDI **1-3**

dissolved in 2 ml of H_2O. The mixture was reacted in the microwave for 30 minutes at 130 °C. The product was filtered, washed with water, and the yield



1 - 3

(1) = CH_3, 89%

(2) = H, 97%

(3) = OH, 96%

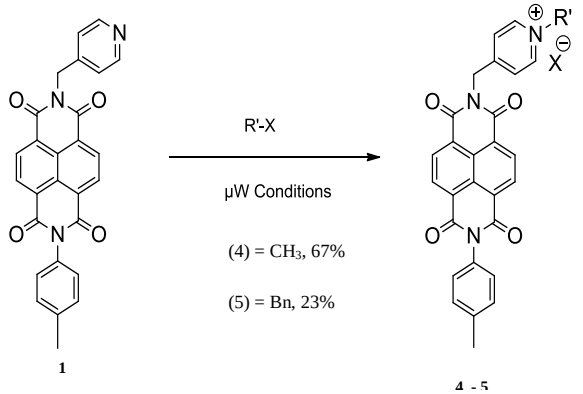
was determined for all three reactions. Proton NMR spectra were obtained for the compounds, using a Bruker 400 MHz NMR spectrometer, to ensure that the asymmetric products were obtained and that they were clean.

Synthesis of alkylated asymmetric NDI 4-8

Asymmetric NDI **1-3** were alkylated to form NDI **4-8**. For the synthesis of **4**, compound **1** was reacted with 10 equivalents of iodomethane under microwave irradiation. The reaction was carried out using 2 ml of acetonitrile as the reaction solvent. The reaction was also carried out using dichloromethane (DCM). The reaction carried out in acetonitrile was reacted in the microwave for 40 min at 140 °C. When DCM was the solvent, the mixture was reacted for 20 min at 80 °C. After removing the products from

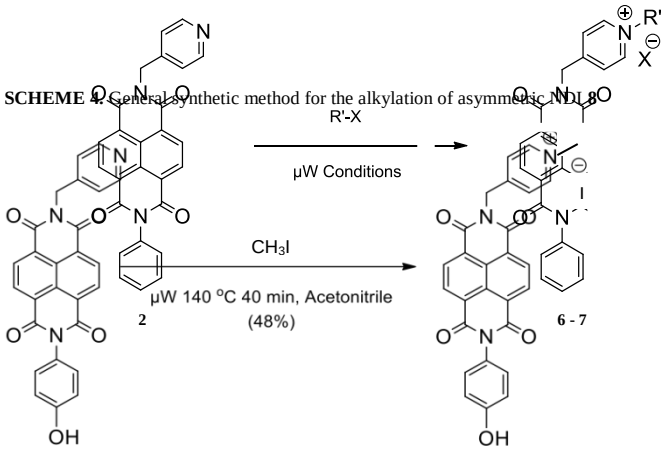
the microwave, they were washed with their respective solvents. Compound **5** was synthesized using 20 equivalents of benzyl bromide, in 2 ml of acetonitrile, at 140 °C for 40 min (SCHEME 2).

SCHEME 2. General synthetic method for the alkylation of asymmetric NDI **4** and **5**



Asymmetric NDI **2** was reacted with 20 equivalents of iodomethane in 2 ml acetonitrile in the microwave at 140 °C for 40 min. The product, **6**, was washed with acetonitrile. The same reaction was also carried out in DMC and acetone. Both were reacted in the microwave for 20 min at 80 °C. The reaction solvents were varied and the reaction was carried out under standard heating conditions. Compound **2** was also alkylated using 20 equivalents of benzyl bromide in DCM and acetone to obtain compound **7**. Both vessels were microwaved for 20 min at 80 °C (SCHEME 3). The products were washed in their respective solvents and their yields were obtained after drying.

SCHEME 3. General synthetic method for the alkylation of asymmetric NDI **6** and **7**



SCHEME 4. General synthetic method for the alkylation of asymmetric NDI **8**

(6) = CH_3, 67%

(7) = Bn, 69%

T

he final NDI, compound **8**, was synthesized by reacting **3** with 20 equivalents of iodomethane in 2 ml of acetonitrile. The reaction was carried out in the microwave at 140 °C for 40 min (SCHEME 4). The product of the reaction was washed with acetonitrile.

After compounds **4**-**8** were washed and allowed to dry, the yields of each product were determined. ^{1}H NMR spectra were obtained for each compound to ensure that the anticipated products were obtained and that they were clean.

DNA Melting Curves

DNA melting experiments were carried out to determine if the synthesized ligands were capable of stabilizing G-quadruplex DNA. Due to time constraints, only compounds **4** and **6** were tested.

Solution Preparation: A 10 mM solution of TRIS-HCl was prepared by adding 1 ml of 1 M TRIS-HCl to a flask. The final volume in the flask was brought to 100 ml through the addition of nano-pure H_2O. A 1 M stock solution of KCl was prepared by dissolving 7.46 g of KCl salt in 100 ml of the 10 mM TRIS-HCl buffer. A 100 μM stock solution was prepared for each compound tested using a serial dilution. Between 10 and 20 mg of the compound were dissolved with a minimal amount of DMSO in a volumetric flask. The final volume was filled to 5 ml with nano-pure H_2O. The human telomerase gene sequence that forms G-quadruplexes (H-Telo 22: 5'AGG GTT AGG GTT AGG GTT AGG3') was purchased with a concentration of 332 μM.

Sample Preparation: Four eppendorf tubes were prepared for each compound. Each contained a final volume of 1 ml. To each of the tubes, 100 μl of the 1 M KCl solution were added so that the final concentration of KCl in the tubes was 100 mM. The HTelo DNA was added in 3 μl aliquots to all four of the tubes, resulting in a final concentration of 1 μM. The amount of compound added to each tube varied. The first tube acted as a control and contained no compound. To the three remaining tubes, 10, 20, and 50 μl of the sample were added to give final concentrations of 1, 2, and 5 μM, respectively. The appropriate amount of buffer was added to each tube to achieve the final volume of 1 ml. The solutions were prepared with sterilized pipette tips and the tubes were vortexed to ensure the final solution was thoroughly mixed. Each solution was added to a labeled and capped quartz cuvette.

Testing Procedure: The melting curves were obtained using a Varian Cary 300 Bio UV-Visible Spectrophotometer with a temperature control system. The cuvettes were inserted into the cells of the spectrophotometer. A cuvette containing only buffer was inserted into a fifth cell. One temperature probe was inserted into the block to monitor its

temperature, and a second into the fifth cuvette to monitor the temperature in the cuvettes. The instrument was set to obtain absorbance readings at 295 nm.[13] The temperature controls were set so that the temperature would immediately increase to 95 °C and decrease to 25 °C at a rate of 1 °C/min. This promoted the melting of the DNA strands followed by the annealing of the strands. Upon reaching 25 °C, the temperature was increased to 95 °C, once more, to promote DNA melting. The software presented the data in the form of a curve that plotted absorbance vs. temperature. Two runs were completed in this manner, for both of the compounds tested.

Analysis of Results: The melting temperature (T_m) is that temperature at which half of the complex is dissociated. On the melting curve, the T_m can be identified as the inflection point. A more accurate method to determine the T_m of the complex was used. The first derivative of the data, obtained from the Thermal UV-Vis software, was plotted in Excel. The maxima of these plots were recorded as the T_m. The mean of the melting temperatures were determined for the two runs. The change in melting temperature (ΔT_m) was determined by calculating the difference in T_m between the DNA control and each of the samples containing the compound under investigation.

Results

Synthesis of asymmetric NDI 1-3

TABLE 1. Identity of R group and yield for the synthesis of NDI **1-3**

Compound	R	% Yield
1	$-CH_3$	89
2	-H	97
3	-OH	96

Synthesis of alkylated asymmetric NDI 4-8

TABLE 2. Identity of alkylating agent and reaction conditions for the synthesis of alkylated NDI **4** and **5**

Compound	R'-X	Solvent	Temperature (^{o}C)	Time (min)	% Yield
4	CH_3I	Acetonitrile	140	40	67
		DCM	80	20	37
5		Acetonitrile	140	40	23

TABLE 3. Identity of alkylating agent and reaction conditions for the synthesis of alkylated NDI **6** and **7**

Compound	R'-X	Solvent	Temperature (^{o}C)	Time (min)	% Yield
6	CH_3I	Acetonitrile	140	40	47
		DCM	80	20	45
		Acetone	80	20	67
7		DCM	80	20	69
		Acetone	80	20	52

TABLE 4. Reaction conditions for the alkylation of NDI **6** using standard heating conditions

Compound	R'-X	Solvent	Time (days)	% Yield
6	CH_3I	Acetonitrile	2	46
		DCM	2	58
		Acetone	2	75

DNA Melting

T_m measurements were obtained for **4** and **6**.

TABLE 5. T_m values obtained for the DNA control and three dilutions of **4** for two runs of the melting experiment

Sample (DNA : Compound 4)	Run 1 T_m (°C)	Run 2 T_m (°C)
DNA	64.0	63.5
1:1	66.0	66.0
1:2	65.5	67.0
1:5	67.0	67.5

TABLE 6. T_m values obtained for the DNA control and three dilutions of **6** for two runs of the melting experiment

Sample (DNA : Compound 6)	Run 1 T_m (°C)	Run 2 T_m (°C)
DNA	64.5	65.0
1:1	68.6	68.4
1:2	68.7	68.0
1:5	70.8	70.5

The ΔT_m was determined for **4** and **6**.

TABLE 7. ΔT_m values obtained from the mean T_m for **4**

DNA : Compound 4	ΔT_m (°C)
1:1	2.2
1:2	2.5
1:5	3.5

TABLE 8. ΔT_m values obtained from the mean T_m for **6**

DNA : Compound 6	ΔT_m (°C)
1:1	3.7
1:2	3.6
1:5	5.9

Discussion

Ligand Synthesis

NDI **1**-**3** were used to assemble potential G-quadruplex stabilizing ligands. The synthetic strategy used to obtain NDI ligands **1**-**3**, as described in SCHEME 1, was accomplished using microwave irradiation in excellent yields (TABLE 1). The compounds were analyzed through ^{1}H NMR and the spectra indicated that the products were clean. Alkylated NDI **4** was synthesized in good yield by reacting asymmetric NDI **1** with iodomethane under microwave irradiation (TABLE 2). The reaction was carried out using two solvents in an attempt to obtain better ^{1}H NMR signals. Hoping to obtain a higher yield, alkylated NDI **5** was synthesized using a substantial excess of benzyl bromide. The reaction conditions resulted in a descent yield (TABLE 2). Both compounds were analyzed through ^{1}H NMR and the spectra indicated that the products were clean. Alkylated NDI **6** and **7** were synthesized in good yield by reacting asymmetric NDI **2** with the alkyl halides under microwave irradiation (TABLE 3). While the yields were sufficient for the purpose of this research, an additional study of the reaction conditions was carried out to ensure that yields were not being sacrificed for shorter reaction times using the microwave. To accomplish this task, the reaction solvents were varied and the yields were compared to those obtained under standard-heating conditions for compound **6** (TABLE 4). This study demonstrated that refluxing provided comparable yields but required significantly longer reaction times. The yields of the refluxing reactions were considered after the comparative microwave times. At the 40 and 20 minute time points, the yield was either minimal or no product had yet formed. These results indicated that the use of microwave irradiation for these reactions was significantly more efficient. The solvents were varied for both reaction setups to identify if there was a significant difference in yield. While the difference was large for the refluxed reaction, the difference was negligible under microwave conditions. Microwave irradiation was also used to alkylate compound **3** to produce compound **8** in good yield (SCHEME 4). This compound was particularly interesting. It was unknown how the hydroxyl functional group would affect the ligand's interaction with the G-quadruplex structure. The compound was determined to be clean based on the ^{1}H NMR spectrum. Future work can be done to further confirm the structures by analyzing the carbon NMR and COSY spectra for the compounds.

DNA Melting Experiments

Due to time constraints, melting experiments were only carried out on compounds **4** and **6**. Future work would involve studying the rest of the compounds using DNA melting experiments. These compounds were selected for testing because they were synthesized in great excess. The results indicated that there was very minor quadruplex stabilization for both compounds. Because of the higher concentration of the compound, it was expected that the 1:5 DNA: compound samples would exhibit the most significant stabilization. Compound **4** resulted in a ΔT_m of 3.5 °C (TABLE 7). The results for **6** were more promising at 5.9 °C (TABLE 8). Based on these results, it is possible that the toluene in **4** may have interfered with ligand-quadruplex interactions. In both cases, the iodide counter ion may have decreased the solubility of the compound. Counter ion exchanges can be carried out in the future to assess this issue. The structures were based off of the information from the literature and showed minor success. It is likely that the large planar core structure allowed for efficient stacking of the ligands in the quadruplex structure. Increasing the size of the core structure, by adding more planar regions, could enhance this stacking. The positive charges seemed to interact with the negative charges as anticipated. The interactions that exist in these systems should be studied further through molecular modeling. Modeling would demonstrate how the ligands interact with the G-quadruplex structure. Neither compound resulted in the significant stabilization observed in previously synthesized ligands from the literature. The results do indicate that the NDI structure should be explored further as a class of potential G-quadruplex stabilizing ligands. These results, though not ideal, met the objectives of the research, which were to use the literature to propose ligand structures, synthesize novel ligands, and test them for G-quadruplex stabilizing capabilities.

Conclusion

Several G-quadruplex stabilizing ligands were synthesized successfully and compounds **4** and **6** were studied to determine their ability to interact with quadruplex DNA. The DNA melting experiments indicated that compounds **4** and **6** exhibit minor G-quadruplex stabilizing capabilities with ΔT_m values of 3.5 and 5.9 °C, respectively. This novel class of NDI derivatives shows potential as G-quadruplex stabilizing ligands and can be greatly expanded through future work.

GENE DELIVERY USING PLGA NANOPARTICLES:

HIGH TRANSFECTION EFFICIENCY WITH LOW TOXICITY

CAROLINE LAY

ABSTRACT

The purpose of this research is to formulate a biodegradable nanoparticle delivery system that has both high transfection efficiency and low cytotoxicity. This will be tested through the transfection of human embryonic kidney cells (HEK 293T) and human colon carcinoma cells (HT29) with a GFP plasmid using PLGA nanoparticles as a carrier system. In addition, chitosan and albumin nanoparticles will be investigated. If effective in both cells, the delivery efficiency of plasmid DNA will be tested in CD8+ cells. Once the gene delivery to the CD8+ cells is successful, shRNA can be delivered to the cells in hopes to silence the GFP protein. The ultimate goal for this project is to silence target genes with shRNA using biodegradable nanoparticles through the use of antibody recognition.

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Introduction

Numerous diseases are determined by genetics. The genes that are inherited from one's parents ultimately establish whether or not one has to live with diseases such as Sickle cell Anemia, Tay Sachs, Huntington's disease, Cystic Fibrosis or any other of the many genetic disorders. However, with recent scientific developments, researchers have been able to target genetically related diseases at the molecular level in hopes to find a cure, known as gene therapy (Sade, Khushf, 1998).

Gene therapy, also identified as gene delivery, is defined as intracellular delivery of genetic material (by transfection) to generate a therapeutic effect by correcting an existing abnormality or providing cells with a new function (Park, 2004). Gene delivery can have many applications, including but not limited to: correcting a mutated gene, replacing a defective or missing gene, and augmenting functions of an existing gene (Park, 2004). The first study that was ever conducted on humans and was recognized took place in 1990. Dr. Michael Blaese, Dr. French Anderson and colleagues performed gene delivery on four year old Ashanti DeSilva, who was treated for ADA-SCID, which is an immune disorder that affects the production of the enzyme adenosine deaminase. The girl's blood was drawn and the gene she lacked was added to her white blood cells (Blaese, R. M., Culver, K. W., Miller, A. D., Carter, C. S., Fleisher, T., Clerici, M., Shearer, G, 1994). The gene therapy was deemed successful, but DeSilva continued to undergo treatment where she received the adenosine deaminase enzyme. Therefore the success cannot be solely attributed to the gene therapy alone because she was receiving normal doses of her medication, as well (Blaese et al, 1994).

In 2001, *Nature* published research that exhibited that gene therapy was successful in slowing the degradation of severe retinal degeneration, Leber congenital amaurosis (LCA). This disease causes near total blindness in infancy and is due to the mutation of the RPE65 gene. Gene therapy was used to introduce the wild-type ("normal") RPE65 gene in order to replace the mutated existing gene in dogs. The gene was successfully delivered to dogs with the disease, and visual function was restored (Acland, Aguirre, Ray, Zhang, Aleman, Cideciyan, Pearce-Kelling, Anand, Zeng, Maguire, Jacobson, Hauswirth, and Bennet, 2001).

Unfortunately, there are many barriers to gene delivery. Intracellularly, the cell and nuclear membrane act as barriers along with the fact that the DNA must be released at the opportune time at the right location (Park, 2004). There are also factors such as the physical and chemical stability of the DNA, and the possible immunogenic response to the vector (Wiethoff, 2002).

A major barrier to gene therapy includes the toxicity of the delivery system used. In the study that focused on enzyme levels in the liver of mice, a positively charged cationic lipid was used to deliver the plasmid DNA, which was injected into the mice intravenously. It was found that the lipid:pDNA complexes triggered significant detrimental changes in the mice, such as leukopenia (low white blood cell count), thrombocytopenia (low thrombocyte levels), and elevated levels of serum transaminases, which indicate liver cell death (Tousignant, Gates, Ingram, Johnson, Nietupski, Cheng, Eastman, Scheule, 2000). However, the exact mechanism of the toxicity is not completely understood. In a review of the study, it was predicted that the toxicity was associated to the net charge of the carrier system (Wiethoff and Middaugh, 2003).

Physical, viral and non-viral methods have been implemented in gene delivery. Physical techniques include direct micro-injection of materials (which is physically introducing DNA into cell using a glass micropipette at a microscopic level), biolistic particle delivery (using a gene gun) and electroporation, which is the use of a high energy electric field (Niidome, Huang, 2002). A study was conducted using rats which tested the efficiency of the electroporation physical gene delivery system. The reporter gene, pEGFP-N1, which codes for green fluorescent protein (GFP) was delivered to the skin and assayed with Confocal Laser Scanning Microscopy (CLSM). The researchers found that the plasmid was able to penetrate into the epidermis within minutes after electroporation, and after hours was able to enter the keratinocyte (cell in the outer most layer of skin) and the cytoplasm (Dujardin, Van Der Smissen, Preat, 2001). The GFP was able to be observed for seven days after the electroporation and the viability of the skin was not compromised during the process. These findings show that the electroporation enhances delivery, causing expression and therefore, can be an alternative approach to administer DNA to the skin (Dujardin et al, 2001).

Viral vectors are also used as gene delivery systems. This system involves genetically altered viruses that have their genome altered to hopefully reduce the chance of viral replication, reduce cytotoxicity, and permit incorporation of the desired gene (Wiethoff and Middaugh, 2002). The vectors used in a viral carrier system include: retroviral vectors, lentriviral vectors, adenoviral vectors, adeno-associated virus (AAV), and herpes simplex viral vectors (HSV). The viral vector systems show patterns of higher gene transfer efficiency than the non-viral gene carrier systems (Park, 2004). However, viral systems also have the potential risk of the regeneration of the wild type virus that is harmful to the body, as well as the danger of immunogenicity, which is when an immune response is activated within the body. In 1998, replication-defective adenoviral (RDAd) vectors were used to inject the leptin gene into mice that had a mutated leptin gene, which caused excessive hunger and desire for food, leading to obesity. The results showed that the adenoviral vectors were able to generate high replication deficient recombinant viruses and have efficient gene transfer to a wide variety of both nondividing and dividing cells (Kafri, Morgan, Krahl, Sarvetnick, Sherman, Verma, 1998). However, there was an immunogenic response by the mice which was most likely due to the adenoviral proteins (Kafri et al, 1998). The mice that experienced the gene therapy injection were noted to show lymphocyte infiltration and cytotoxic-T- lymphocyte lysis, which supports that the mice recognized the virus as foreign and had an immunogenic response to the viral vectors (Kafri et al, 1998).

Nonviral vector delivery systems include naked DNA delivery by a physical method and delivery mediated by a chemical carrier, such as a cationic polymer (Niidome and Huang, 2002). There are many benefits to nonviral delivery systems when compared to the viral technique. Firstly, there the molecular composition can be controlled for simplified manufacturing and analysis. Also, there is less of a chance of immunogenic response (Wiethoff and Middaugh, 2002). Since the discovery of transfection, many different approaches have been employed. Calcium phosphate precipitation and diethylaminoethyl-dextran transfection were two of the earlier methods developed for the delivery of DNA (Nikcevi, Kovacevic-Gruijicic, Stevanovic; 2003). The calcium phosphate precipitation technique uses a solution of calcium chloride mixed with the plasmid DNA which is added to a phosphate-buffered solution. The DEAE-dextran transfection uses dimethyl sulfoxide to increase DNA uptake from

the cells within a DNA/DEAE-dextran mixture**.** Another method is known as cationic liposomes, which is thought to be better endured by the cells (Nikcevi et al, 2003). This process works because the cationic liposomes interact with the negatively charged nucleic acid molecules and the DNA-bound lipids associate with the cell membrane, causing DNA internalization to occur (Nikcevi et al, 2003).

Nanoparticles prepared from biodegradable polymers are being expansively explored as non-viral gene delivery systems because of their persistent release features and biocompatibility (Prabha, Zhou, Panyam, Labhasetwar, 2002). Because of the small size of the nanoparticle, they are able to be successfully endocytosed by the cells which result in higher cellular uptake of the entrapped DNA. The DNA is encapsulated within the polymeric matrix, so it is secure from both intracellular and extracellular nuclease degradation (Prabha et al, 2002). When the polymer matrix is hydrolyzed, the ester bonds are cleaved, which release the DNA inside the nanoparticles (Prabha et al, 2002). A major factor that affects the transfection efficiency of the nanoparticle is its size. The size of the particle has great repercussions on their cellular and tissue uptake and the smaller particles (nanoparticles) have shown greater transfection results as opposed to the larger ones (microparticles) (Prabha et al, 2002). The benefits of using this type of delivery system is that it has high stability/shelf life, high carrier capacity, incorporation of both hydrophilic and hydrophobic substances, and variable routes of administration (Gelperina, Kisich, Iseman and Heifets, 2005). According to research focusing on the delivery of siRNA into targeted 293T cells in order to silence GFP, it was found that "nanoparticles offered both effective delivery of siRNA and prominent GFP gene silencing effect. Compared to conventional carrier systems, the new biodegradable polymeric nanoparticle system may also offer improved formulation stability" (Yuan, Li, Rathinavelu, Hao, He, Heitlage, Tam, Viqar, and Salehi, 2006).

RNA interference (RNAi) is a natural process in which expression of a targeted gene can be inactivated, or silenced, through the use of small double stranded RNA (Rao, Vorhies, Senzer, Nemunatis, 2009). Through gene delivery, short interfering RNA (siRNA), short hairpin RNA (shRNA) and plasmid DNA (pDNA) can be introduced into cells in order to suppress an existing gene that is defective. Short interfering RNA can be synthetically produced and can be directly introduced into cells in order to

silence genes. They are usually 21-23 nucleotides long and are double stranded RNA molecules. These RNAs complex with the RNA-induced silencing complex (RISC protein), forming a complex which is able to degrade and cleave the recognized mRNA of the cell. Short hairpin RNA is a different from siRNA. It is transcribed as short hairpin precursors which are usually around 70 nucleotides long. Dicer, which is an RNase III enzyme, cleaves the shRNA into its active 21-nucleotide form which can then silence the expression of specific genes (Rao et al, 2002). As opposed to the siRNA, shRNA needs to be cloned into a vector in order to be delivered into the cell. However, once inside the cell, it can be synthesized by the host cell, which proves more efficient and more durable (Rao et al, 2002). In the "Advanced Drug Delivery Reviews on siRNA vs. shRNA", Donald Rao and his colleges discuss the benefits and disadvantages of working with these RNAs. The researchers explain that chemically synthesized siRNA is easier to alter through chemical practices. However, it is more expensive. To the contrary, vector based shRNA relies on the host machinery for its expression, but is more challenging to modify (Rao et al, 2002). RNA interference can be applied to gene therapy to aim to silence an existing mutated gene.

Objectives

The purpose of this research is to successfully transfect 293T cells and HT29 cells with a GFP plasmid using the PLGA nanoparticles as a carrier system. If this is effective, the cell type will change in order to test the delivery efficiency using CD8+ cells. Once the delivery system to the CD8+ cells is successful, shRNA will be delivered to the cells in hopes to silence the GFP protein.

Materials and Methods

Culturing of 293T cells

The Human Embryonic Kidney 293 cells (293T) were sent by ATCC. Upon arrival, the cells were thawed in a 37^0C water bath for approximately two minutes. The cell vial was then disinfected using ethanol. The cells were then spun down in the centrifuge at 200G for approximately 4 minutes. Once a pellet was observed, the supernatant was decanted into a cell flask (25

cm^2) labeled "293 T – supernatant" with 10 ml of culture medium (Dulbecco's Modified Eagle's Medium, 10% FBS with penicillin/streptomycin). The pellet was then resuspended in culture medium then transferred into a cell flask (25 cm^2). Approximately 10 ml of culture medium was added to the flask labeled "293 T – cells). Both flasks were placed in the incubator at 37^0C and 5% Carbon Dioxide. The cells were observed every 1-2 days and subcultured as needed.

Subculture Protocol

When the cells reached 80-90% confluency, they were subcultured in to multiple flasks. Once the cells were visualized under microscope and cell attachment to flask was confirmed, the culture medium was removed. The cells were then washed with PBS (Phosphate-Buffered Saline) in order to remove any debris. The PBS was decanted from the flask and 2 ml of 0.25% Trypsin-EDTA solution was added to the flask. The flask was placed in the incubator for approximately 3 minutes. Upon removal from the incubator, the cells were observed under the microscope to confirm separation from flask. Approximately 10 ml of culture medium was added to cease the trypsinization process. The contents of the flasks where then transferred equally into 3 new flasks (25 cm^2). Culture medium was then added to all of the flasks to maintain the maximum content level of 10 ml. The cells were observed every 1-2 days.

Culturing HT 29 Cells

The Human Colon Carcinoma cell line (HT29) was sent by ATCC. Upon arrival, the cells were thawed in a 37^0C water bath for approximately two minutes. The cell vial was then disinfected using ethanol. The cells were then spun down in the centrifuge at 200G for approximately 4 minutes. Once a pellet was observed, the supernatant was decanted into a cell flask (25 cm^2) labeled "HT29 – supernatant" with 10 ml of culture medium (McCoys 5A Medium with 1.5 mM L-glutamine and 10% Fetal Bovine Serum). The pellet was then resuspended in culture medium then transferred into a cell flask (25 cm^2). Approximately 10 ml of culture medium was added to the flask labeled "HT29 – cells). Both flasks were placed in the incubator at 37^0C and 5% Carbon Dioxide. The cells were observed every 1-2 days and subcultured as needed.

Lipofectamine transfection of GFP plasmid DNA of 293T cells

The 293T cells were counted with hemocytometer in order to properly prepare cell suspension with cell density of 10^5 cells/ml per well. One

milliliter of cells were then transferred to the wells of a 12-count well plate along with 2 ml of culture medium (DMEM, 10% FBS, and antibiotics). After approximately 24 hours, the culture medium was replaced with antibiotic-free medium. Then, Mixture A was prepared: GFP plasmid (5 µg, 10 µg, 25 µg, and 50 µg) and Opti-MEM to obtain a total of 50 µl per well. The mixture was vortexed and allowed to sit for 5 minutes. Mixture B was prepared: 2 µl of Lipofectamine with 48 µl Opti-MEM, vortexed, then allowed to sit for 5 minutes. Mixture A and B were then mixed and vortexed 3 times then allowed to sit for 20 minutes. One hundred microliters of mixture was then suspended into each well and incubated for 4 hours. Then, fluorescence displayed by the GFP plasmid DNA was observed under the fluorescent microscope.

Lipofectamine transfection of GFP plasmid DNA of HT29 cells

The HT29 cells were counted with hemocytometer in order to properly prepare cell suspension with cell density of 10^5 cells/ml per well. One milliliter of cells were then transferred to the wells of a 12-count well plate along with 2 ml of culture medium (McCoy's Medium with 10% FBS). The cells were allowed to attach to the bottom of the wells in a 24 hour incubation period. Then, Mixture A was prepared: GFP plasmid DNA (5 µg, 10 µg, 25 µg, and 50 µg) and Opti-MEM to obtain a total of 50 µl per well. The mixture was vortexed and allowed to sit for 5 minutes. Mixture B was prepared: 2 µl of Lipofectamine with 48 µl Opti-MEM, vortexed, then allowed to sit for 5 minutes. Mixture A and B were then mixed and vortexed 3 times then allowed to sit for 20 minutes. One hundred microliters of mixture was then suspended into each well and incubated for 4 hours. Then, GFP fluorescence was observed under the fluorescent microscope.

Delivery of GFP Plasmid DNA using PLGA Nanoparticles to Human Embryonic Kidney cells (293T)

293T cells were removed from a 25cm^2 flask through trypsinitzation and plated in a

12-count well plate with 1 ml of cells (approximately 10^5 cells per ml) and 2 ml of culture medium (DMEM with 10% FBS and antibiotics) per well. The well plate was incubated for 24 hours to allow cells to adhere to the bottom of the flask. The existing culture medium was carefully removed with an automatic pipet and new medium lacking antibiotics was then added and the plate was incubated for 3 hours. During the

incubation period, the nanoparticles were prepared. All of the glassware was autoclaved to ensure sterility. Two hundred and forty milligrams of Polyvinyl Alcohol (PVA) was weighed and dissolved in 12 ml of distilled water using a glass stirring rod. Then, 60 mg of Poly(lactic-co-glycolic acid) was measured and dissolved in 9 ml of acetone and 3 ml of ethanol. The GFP plasmid DNA was thawed and 10 µl was added to a small microcentrifuge tube and diluted using 40 ml of distilled water. The PVA solution that was previously prepared was then evenly divided into 3 beakers and the GFP plasmid DNA was added in the amounts of 1 µg, 2 µg, and 5 µg to each correspondingly labeled beaker. Then, 4 ml of the Poly(lactic-co-glycolic acid) solution was added to each beaker drop wise using a 5 ml syringe. The organic solvent was allowed to evaporate for approximately 25 minutes. The solutions in each beaker were then split into 4 microcentrifuge tubes (approximately 1 ml each), and centrifuged at 12,000 rpm and 4^{o}C for 15 minutes. The supernatant was transferred into new microcentrifuge tubes and then centrifuged again at 15,000 rpm and 4^{o}C for 30 minutes. The supernatant was discarded. The pellet was resuspended using 100 µl of Opti-Mem, and then transferred into the microcentrifuge tube containing the original pellet, which was also resuspended. Then, 1 ml of each tube was added to the corresponding well of 293T cells and allowed to incubate for 24 hours. The following day, the cells from each well were transferred to a fluorescent microscope slide by physically detaching the cells through constant pipetting, and observed under the fluorescent microscope to detect fluorescence.

Delivery of GFP Plasmid DNA using PLGA Nanoparticles to Human Colon Carcinoma Cells (HT29)

HT29 cells were removed from a 25cm^2 flask through trypsinitzation and plated in a 12-count well plate with 1 ml of cells (approximately 10^5 cells per ml) and 2 ml of culture medium (McCoy's Medium with 10% FBS). The well plate was incubated for 24 hours to allow cells to adhere to the bottom of the flask. The following day, all of the glassware was autoclaved to ensure sterility. Two hundred and forty milligrams of Polyvinyl Alcohol (PVA) was weighed and dissolved in 12 ml of distilled water using a glass stirring rod. Then, 60 mg of Poly(lactic-co-glycolic acid) was measured and dissolved in 9 ml of acetone and 3 ml of ethanol. The GFP plasmid DNA was thawed and 10

µl was added to a small microcentrifuge tube and diluted using 40 ml of distilled water. The PVA solution that was previously prepared was then evenly divided into 3 beakers and the GFP plasmid DNA was added in the amounts of 1 µg, 2 µg, and 5 µg to each correspondingly labeled beaker. Then, 4 ml of the Poly(lactic-co-glycolic acid) solution was added to each beaker drop wise using a 5 ml syringe. The organic solvent was allowed to evaporate for approximately 25 minutes. The solutions in each beaker were then split into 4 microcentrifuge tubes (approximately 1 ml each), and centrifuged for 15 minutes at 12,000 rpm and 4^{o}C. The supernatant was transferred into new microcentrifuge tubes and then centrifuged again for 30 minutes at 15,000 rpm and 4^{o}C. The supernatant was discarded. The pellet was resuspended using 100 µl of Opti-Mem, and then transferred into the original pellet, which was also resuspended. Then, 1 ml of each tube was added to the corresponding well of 293 T cells and allowed to incubate for 24 hours. The following day, the cells from each well were transferred on a fluorescent microscope slide by physically removing the cells through constant pipetting, and observed under the fluorescent microscope to detect fluorescence.

XTT Cytotoxicty Assay of Delivery Systems on 293T cells and HT29 cells

The cells were trypinsized in a 25 cm^2 flasks and transferred to a 96-well plate with 100 µl of growth medium per well, and incubated at 37^{o}C and 5% CO_2 for 24 hours. A control row for each cell type was established which did not contain any cells, but only 100 µl culture medium. Transfection was performed (of Lipofectamine, PLGA (with and without PEI), Chitosan, and Albumin (both with and without PEI and PLL). The XTT reagent solution and activation solution (from XTT kit) was placed in a 37^{o}C water bath to thaw. Using a micropipette, 100 µl of activation solution was added to 5 ml of the XTT reagent. Then, 50 µl of the reaction solution was added to each well and incubated for 3 hours. The plate was removed and gently agitated to distribute the orange coloring. The absorbance values were measured using a microplate reader at wavelengths of 450 nm and 655 nm. To calculate the cell viability, the absorbance values for each trial were averaged, then the 655 nm value (background noise), was subtracted from the 450

nm values to get the true absorbance value, representing the cell viability.

Results

Lipofectamine transfection of GFP plasmid DNA of 293T cells

The Human Embryonic Kidney cells were successfully transfected with the GFP plasmid DNA using the Lipofectamine delivery system. The cells displayed an observable fluorescence when viewing under the fluorescent light microscope. The transfection efficiency was measured using varying concentrations (5 µg, 10 µg, 25 µg, and 50 µg) of the plasmid DNA. Because a FACS machine was not available, the amount of fluorescence displayed by the cells was estimated by visual inspection by the researcher. The cells in the wells containing 5 µg of the GFP plasmid displayed an approximate 50% efficiency (Figure 1A). There was a less amount of fluorescence in the 10 µg wells (40%)(Figure 1B) , and even less in the 25 µg well (30%)(Figure 1C). The amount of fluorescence was the lowest in the 50 µg of plasmid DNA; only 25% of the cells displayed fluorescence (Figure 1D).

Lipofectamine transfection of GFP plasmid DNA of HT29 cells

Fluorescence was observed in the Human Colon Carcinoma (HT29) cells using the Lipofectamine transfection of the GFP plasmid DNA. Similar to the 293T cells, GFP was added in concentrations of 5 µg, 10 µg, 25 µg, and 50 µg. Approximately 30% of the cells in the wells containing 5 µg of DNA displayed fluorescence (Figure 2A), whereas only 25% of the cells in the wells containing 10 µg of DNA fluoresced (Figure 2B). There was less fluorescence observed for the 25 µg concentration of plasmid (20%)(Figure 2C), and even less for the 50 µg plasmid concentration, which hardly showed any fluorescence (5%)(Figure 2D).

Delivery of GFP Plasmid DNA using PLGA Nanoparticles to Human Embryonic Kidney cells

The delivery of the GFP plasmid DNA using PLGA nanoparticles was effective in the Human Embryonic Kidney cells. Figure 3A shows the 293T cells that displayed fluorescence after 1 µg of GFP plasmid was delivered into the cells through the PLGA nanoparticle, which showed the

strongest amount of fluorescence among the varying concentrations. Approximately 50% of these cells showed green fluorescence. The wells containing 2 μg of GFP did show a considerable amount of fluorescence (40%)(Figure 3B), but still less than the 1 μg of GFP. Lastly, the well containing 5 μg of the plasmid DNA was positive for fluorescence, but a very small amount (roughly 25%)(Figure 3C).

Delivery of GFP Plasmid DNA using PLGA Nanoparticles to Human Colon Carcinoma Cells

The PLGA nanoparticles were successful in delivering the GFP plasmid DNA to the Human Colon Carcinoma cells. Fluorescence was observed in all of the varying concentrations of plasmid (1 μg, 2 μg, and 5 μg), however it showed the highest efficiency in the 1 μg concentration (70%)(Figure 4A). There was an estimated 50% efficiency of cell fluorescence in the 2 μg concentration of GFP in the HT29 cells (Figure 4B), and a 25% efficiency of cell fluorescence in the 5 μg concentration of GFP.

XTT Cytotoxicty Assay of Delivery Systems Effect on 293T cells

The cytotoxicity results show that the Lipofectamine control, at both 5 μg and 10 μg, resulted in 30% of the 293T cells dying, hence a 70% cell viability rate (table 1). The PLGA nanoparticles had an identical result; 70% of the 293T cells remained living following the transfection of 5 μg of GFP plasmid DNA. When using PLGA with the addition of PEI 0.01% and 0.05% (Polyethylenimine) the viability greatly reduced to 30% and 50%, respectively (table 1). The Chitosan nanoparticles killed the majority of 293T cells, leaving 10% viable. Lastly, the Albumin nanoparticles with PEI (0.01%) transfection had 70% cell sustainability and Albumin nanoparticles with PLL transfection (poly-L-lysine) resulted in half of the cells death (50%) (table 1).

XTT Cytotoxicty Assay of Delivery System Effect on HT29 cells

The 5 μg Lipofectamine control allowed for between 40-60% of HT29 cells to survive, were as the 10 μg Lipofectamine control allowed for slightly less: 40-50% cell viability (table 1). The PLGA had a very high cell viability of 95% and the addition of PEI (0.01% and 0.05%) caused a lower

viability amount (70% and 60%, respectively)(table 1). The Chitosan transfection had a small HT29 survival rate of only 10% (table 1). Lastly, the Albumin with PEI allowed for 80% of the cells to survive, and 75% of the HT29 cells survived the Albumin with PLL transfection (table 1).

Discussion

The transfection of Lipofectamine was established as a control to compare to the PLGA nanoparticle transfection. It has been used in past experimental efforts and deemed a successful mechanism for transfection, so it was expected to work in both cell types. This was verified in the results. The 293T cells were successfully transfected with the plasmid DNA because they displayed fluorescence under the microscope. When comparing the different concentrations of plasmid DNA that were transfected into the cell, it was evident that the fluorescence expression decreased as the concentration increased in the 293T cells (Figure 1-4). For instance, half of the cells that were transfected with the 5 μg concentration displayed fluorescence, whereas only a quarter of cells that were transfected with 50 μg expressed fluorescence. This could be an effect of the excess plasmid DNA being toxic to the cells. This is supported by the cytoxicity results that revealed a higher GFP plasmid DNA concentration (10 μg), there was a 40-50% cell vitality, compared to the 40-60% of the lesser concentration (5 μg). Unfortunately, due to the lack of a FACS (which quantifies fluorescence) machine, the fluorescence expression had to be visually inspected and estimated by the researcher.

When analyzing the results of the Lipofectamine transfection of the GFP plasmid DNA to the Human Colon Carcinoma cells, the transfection was much less effective than the 293T cell transfection, meaning the intensity and amount of fluorescence displayed by the cells was significantly less. This was expected due to the general properties of the cells lines. The Human Embryonic Kidney cells are much more stable and will uptake nutrients more aggressively compared to the HT29 cells. However, a similar relationship can be observed in the HT29 cells as was seen in the 293T cells: as the concentration of GFP plasmid DNA was increased, the transfection efficiency decreased. For example, 30% of the HT29 cells that were transfected with 5 μg of DNA showed fluorescence, whereas the 10 μg, 25

μg, and 50 μg showed 25%, 20%, and 5% transfection efficiency, respectively (Figure 2).

The PLGA nanoparticle delivery of the GFP plasmid DNA was also effective in both cell types. In comparison to the Lipofectamine control transfection, the PLGA transfection had a higher efficiency in the HT29 cells than the 293T cells. This was not expected because, as mentioned early, the HT29 cells are more fragile than the 293T cells and are less likely to uptake nutrients are well as the 293T cells. The PLGA delivery resulted in 50% of the 293T cells displaying fluorescence and as many as 70% of the HT29 cells displaying fluorescence. When analyzing the results of the 293T cells, 1 μg of plasmid DNA resulted in 50% transfection (Figure 3A). The transfection effectiveness was less for the 2 μg and decreased 10% and was only 40% effective (Figure 3B). Lastly, the 5 μg of GFP plasmid DNA resulted in a small 25% transfection proficiency (Figure 3C).

When comparing the two cell types for the PLGA nanoparticle delivery, the HT29 cells resulted in a higher transfection production. This was not expected due to the general properties of each cell type. The 293T cells, as seen in the Lipofectamine delivery, are more aggressive with the uptake of nutrients compared to the HT29 cells. When 1 μg of plasmid DNA was transfected into the HT29 cells, approximately 70% of the cells displayed fluorescence (Figure 4A). For the HT29 cells that were transfected with 2 μg of DNA, approximately half of them fluoresced (Figure 4B), and lastly for the 5 μg of plasmid transfection, only a quarter of the cells fluoresced (Figure 4C). This supports the relationship that as the concentration of plasmid DNA increases, the efficiency of the transfection decrease. When polyethylenimine was added to the PLGA, the transfection efficiency increased in the 293T cell from 50% to 70%. However, this increase in efficiency was not observed in the HT29 cells. When PEI was added to the PLGA, the cell transfection efficiency decreased form 70% to 30% (for 0.01% PEI).

A variety of control delivery systems that have been established in past research were utilized in order to compare the results obtained from the PLGA nanoparticle delivery. Chitosan, which is a cationic polysaccharide obtained from naturally occurring chitin in crustaceans, was used as the first control (*Illum, Farraj, Davis, 1994*). It has been previously explored as a drug delivery system due to its non-toxicity and bioadhesive effect (*Illum et*

al, 1994). When used to deliver GFP plasmid DNA to 293T cells, there was only a 30% effectiveness obtained. This transfection efficiency was matched in the HT29 cells, as well.

Albumin, which has also been used in previous nanoparticle delivery experiments, was also used as a positive control to compare the PLGA delivery to. Albumin is a negatively charged plasma protein that is important in the osmotic regulation (Quinlan, Martin, Evans, 2005). It was tested with polyethylenimine (PEI), and with poly-L-lysine (PLL), both of which are polymeric transfection agents. The transfection efficiency was higher using the PEI (0.01%) which was estimated to be 50%, compared to the transfection with Albumin and PLL which was assessed to be 40% (table 1).

The XTT Cell Proliferation Assay kit was used to determine the cytotoxicity of the PLGA nanoparticles. Other controls were included for comparison (Lipofectamine, Chitosan, and Albumin). The absorbance values were acquired from the microplate reader (at 450 nm and 655 nm) after the addition of the reaction reagents from the kit to determine the cell viability, or survival rate, after the transfection process. The results showed that the PLGA nanoparticles were not very harmful to the vitality of the cells. Approximately 70% of the 293T cells and 95% of the HT29 cells survived the transfection (table 1). This is the highest cell viability compared to all of the other controls ran. The Lipofectamine transfection allowed for the survival of 70% of the 293T and approximately 50% of the HT29 cells. When 0.01% of PEI was added, the cytotoxicity decreased dramatically to 30% for the 293T cells (table 1). When the amount was increased to 0.05% PEI, the cell viability surprisingly went up to 50%. This was not predicted because as the concentration of a harmful solution increases, the amount of cell viability should decrease. This, however, was depicted by the HT29 cells. When PLGA with 0.01% PEI was assayed, there was a cell viability of 70% (table 1). When the cytotoxicity was evaluated using PLGA with 0.05% PEI, the cell viability decreased to 60% (table 1). The interesting results of PEI toxicity can be further investigated in future studies.

The cell viability of the cells after chitosan delivery of the GFP plasmid was very low. Only 10% of both the 293T cells and HT29 remained living following the cytotoxicity assay (table 1). This was very surprising because

Chitosan is both biodegradable and biocompatible (*Illum et al, 1994)*. The viability of the cells could have been so low due tohigh concentration of chitosan and possible contamination of microorganisms, which could have had an effect on the viability of cells. When analyzing the cytotoxicity results of the Albumin delivery system, the cells remained slightly more viable after the Albumin/PEI delivery as opposed to the Albumin/PLL, but the differences between the two was not very drastic. The Albumin/PEI delivery had a 70% cell survival rate in 293T cells and 80% cell viability in HT29. The Albumin/PLL allowed half of the 293T cells to survive, and 75% of the HT29 cells to survive (table 1). Compared to the PLGA, the Albumin nanoparticles resulted in a lower cell viability (table 1).

Conclusion

After analysis of the results, it can be concluded that the PLGA nanoparticles are a successful delivery system of the GFP plasmid DNA. This carrier system has both a high transfection efficiency and a low toxicity. Compared to the other previously studied delivery controls (Lipofectamine, Chitosan, and Albumin), PLGA has the highest efficiency of delivering the GFP plasmid DNA and also as the least detrimental effects to both cell lines studied.

Some limitations to the research that could be improved upon in the future are to use a FACS, or fluorescent-activating cell sorting machine, chambered slides, and an inverted fluorescent microscope. The FACS machine would allow for the fluorescence displayed by the cells to be measured and quantified. This would give a more accurate value to the GFP plasmid transfection efficiency, as opposed to the estimation made by the researcher based on visual observation. The use an inverted fluorescent microscope would prove beneficial because the cells would not have to be transferred after culture and transfection. In the current research, the cells delivery of the DNA took place in a well plate and then the cells were transferred to microscope slides. This posed a problem because it is possible that not all of the cells were transferred, or perhaps a group of non-fluorescing cells were moved to the microscope slide if the cells are not mixed homogenously.

Figures/Tables

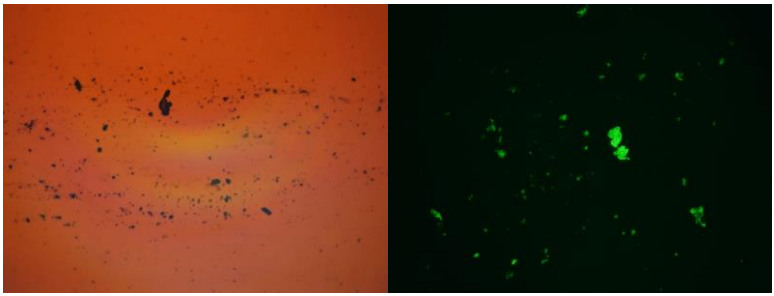

Figure 1A: ***Results of 5 µg of GFP plasmid DNA delivered to 293T cells using Lipofectamine Control***. The left side shows the 293T cells under the bright field microscope setting and the right shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. When estimating the efficiency of the delivery, approximately 50% of the cells displayed fluorescence.

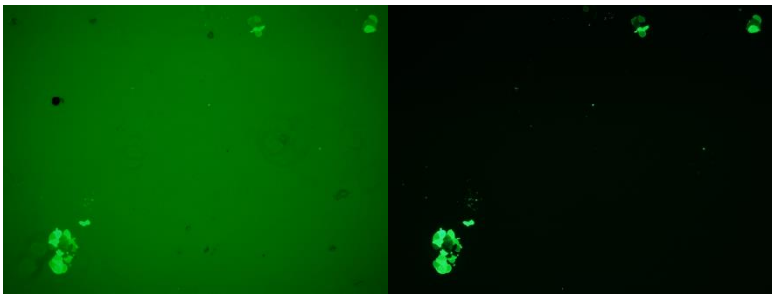

Figure 1B: ***Results of 10 µg of GFP plasmid DNA delivered to 293T cells using Lipofectamine Control***. The left side shows the 293T cells under the bright field microscope setting (with a green filter) and the right shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. Approximately 40% of the 293T cells expressed fluorescence.

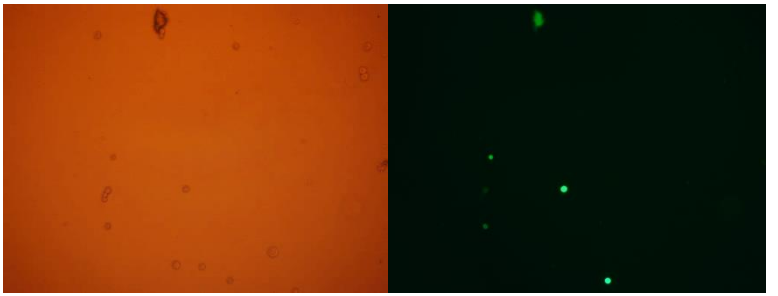

Figure 1C: ***Results of 25 µg of GFP plasmid DNA delivered to 293T cells using Lipofectamine Control***. The left side shows the 293T cells under the bright field microscope setting and the right shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. Approximately 30% of the 293T cells expressed fluorescence.

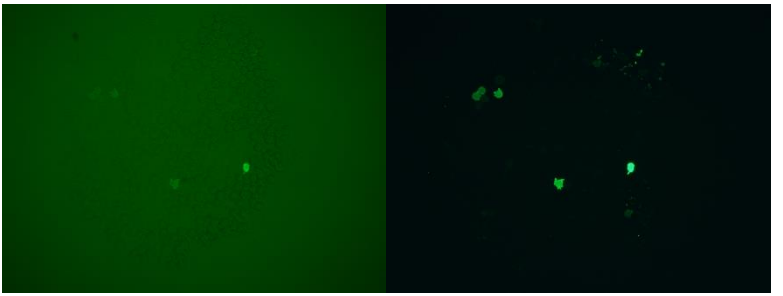

Figure 1D: ***Results of 50 µg of GFP plasmid DNA delivered to 293T cells using Lipofectamine Control***. The left side shows the 293T cells under the bright field microscope setting (with a green filter) and the right shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. Approximately 25% of the 293T cells expressed fluorescence.

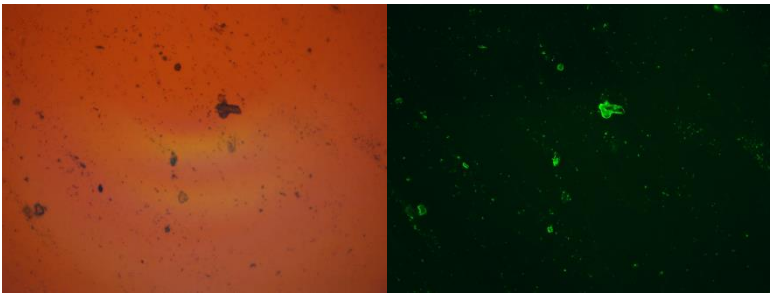

Figure 2A: ***Results of 5 µg of GFP plasmid DNA delivered to HT29 cells using Lipofectamine Control***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 30% of the HT29 cells expressed fluorescence.

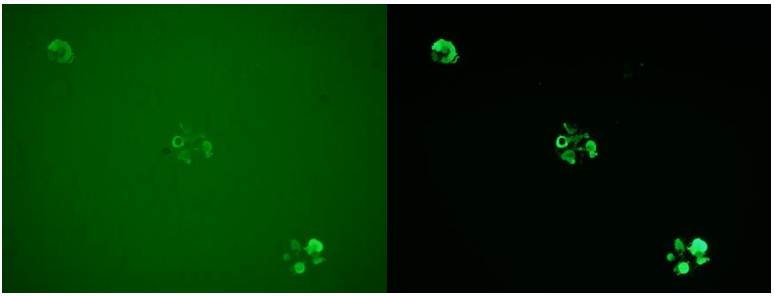

Figure 2B: ***Results of 10 µg of GFP plasmid DNA delivered to HT29 cells using Lipofectamine Control***. The left image shows the HT29 cells under the bright field microscope setting (with a green filter applied) and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 25% of the HT29 cells expressed fluorescence.

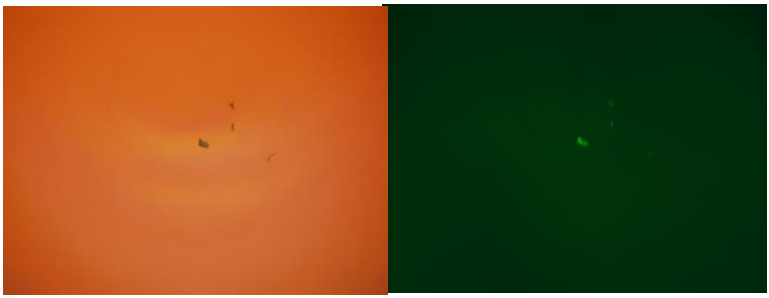

Figure 2C: ***Results of 25 µg of GFP plasmid DNA delivered to HT29 cells using Lipofectamine Control***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 20% of the HT29 cells expressed fluorescence.

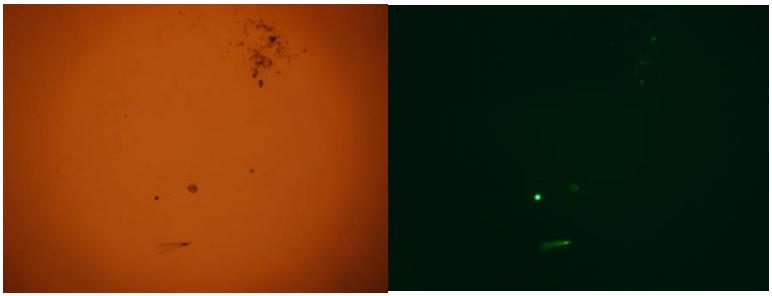

Figure 2D: ***Results of 50 µg of GFP plasmid DNA delivered to HT29 cells using Lipofectamine Control***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. The majority of the cells did not fluorescence, an estimated 5% of the HT29 cells did express fluorescence.

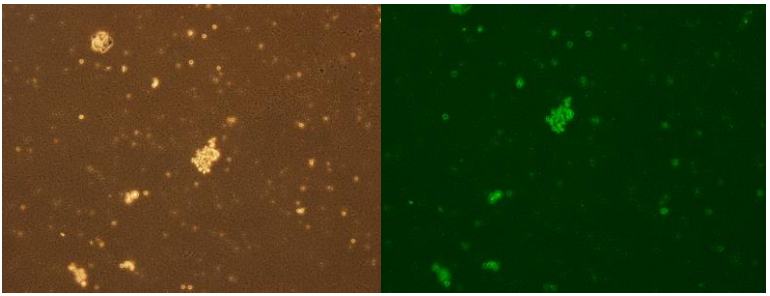

Figure 3A: ***Results of 1 µg of GFP plasmid DNA delivered to 293T cells using PLGA nanoparticle delivery***. The left image shows the 293T cells under the bright field microscope setting and the right image shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 50% of the 293T cells expressed fluorescence.

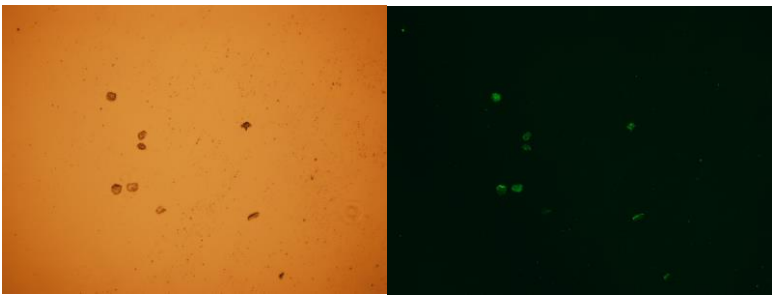

Figure 3B: ***Results of 2 µg of GFP plasmid DNA delivered to 293T cells using PLGA nanoparticle delivery***. The left image shows the 293T cells under the bright field microscope setting and the right image shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 40% of the 293T cells expressed fluorescence.

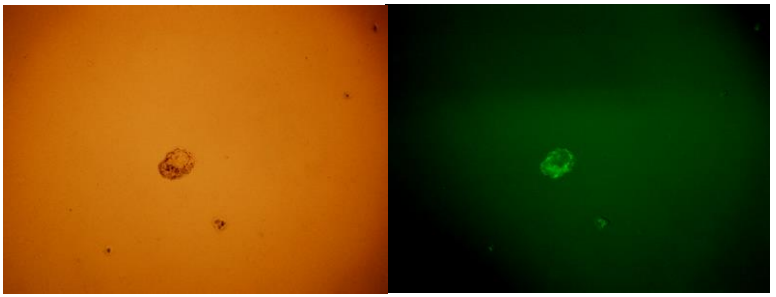

Figure 3C: ***Results of 5 µg of GFP plasmid DNA delivered to 293T cells using PLGA nanoparticle delivery***. The left image shows the 293T cells under the bright field microscope setting and the right image shows 293T cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 25% of the 293T cells expressed fluorescence.

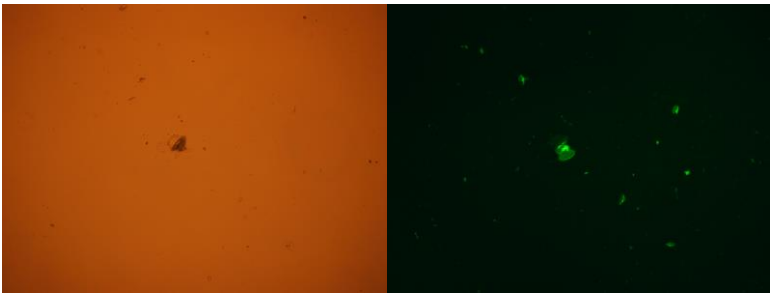

Figure 4A: ***Results of 1 µg of GFP plasmid DNA delivered to HT29 cells using PLGA nanoparticle delivery***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 70% of the HT29 cells expressed fluorescence.

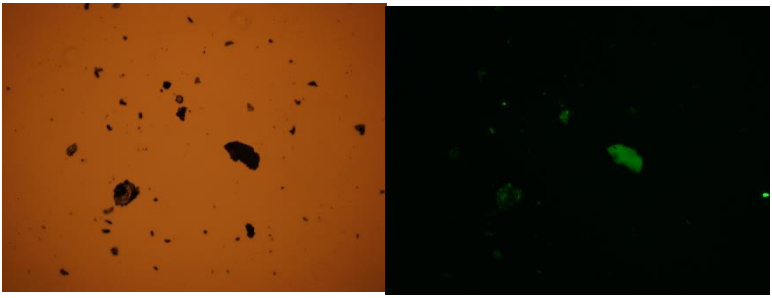

Figure 4B: ***Results of 2 µg of GFP plasmid DNA delivered to HT29 cells using PLGA nanoparticle delivery***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 50% of the HT29 cells expressed fluorescence.

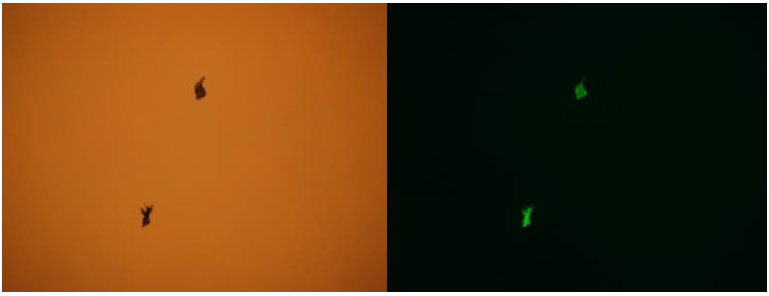

Figure 4C: ***Results of 5 µg of GFP plasmid DNA delivered to HT29 cells using PLGA nanoparticle delivery***. The left image shows the HT29 cells under the bright field microscope setting and the right image shows HT29 cells after the fluorescent filter was applied. The expression of the green fluorescence shows a successful delivery of the GFP plasmid DNA. An estimated 25% of the HT29 cells expressed fluorescence.

Table 1: ***The XTT Cell Proliferation Assay Results and Cell Transfection Efficiency Estimations.*** This table displays information from all of the delivery systems used in the research to deliver the GFP plasmid DNA to 293T cells (left side) and HT29 cells (right side). The amount of plasmid DNA (μg) is provided, as well as the estimated transfection efficiencies and the calculated cell viability.

293T cells				HT29 cells			
Sample	GFP Plasmid (μg)	% cell GFP transfection	% cell viability	Sample	GFP plasmid (μg)	% cell GFP transfection	% cell viability
Lipofectamine Control	5	80	70	Lipofectamine Control	5	30	40-60
	10	70	70		10	20	40-50
PLGA	5	50	70	PLGA	5	70	95
PLGA/0.01% PEI	3	70	30	PLGA/0.01% PEI	3	30	70
PLGA/0.05% PEI	5	70	50	PLGA/0.05% PEI	5	40	60
Chitosan	3	30	10	Chitosan	3	30	10
Albumin/0.01 % PEI	3	50	70	Albumin/0.01 %PEI	3	50	80
Albumin/PLL	3	40	50	Albumin/PLL	3	20	75

Scansioned Music: A Glenn Gould Collection

BY JENNIFER VAN ALSTYNE
Creative Writing

For Edwin B. Schneider
1933-2007

Statement of Aesthetics

I never thought that I would be a writer, much less be a poet. Much to my family's initial disappointment, I found myself drawn to language more than anything else. I read everything from 'beach trash' to literary non-fiction and my style has been influenced by everyone from Michael Connolly to Bill Bryson to Daphne du Maurier, none of which, I might note, are poets. For me, poetry is a series of moments which deserve to be frozen. As du Maurier wrote, "If only there could be an invention that bottled up a memory, like scent. And it never faded, and it never got stale. And then, when one wanted it, the bottle could be uncorked, and it would be like living the moment all over again." Some of these moments aren't happy, or good, or important, but that doesn't make them any less worthy of writing about. Glenn Gould is one of the greatest and most influential musicians of all time and he deserves to be remembered. In this collection, I have intertwined his life, memories, and moments of intrigue, with my own. Gould believed that there were many forms of music, from words to notes to sounds. This then becomes my composition for him.

CONTENTS

VARIATIONS

VARIATIONS

I tend to follow a very nocturnal sort of existence mainly because I don't much care for sunlight. Bright colors of any kind depress me, in fact. & my moods are more or less inversely related to the clarity of the sky, on any given day. A matter of fact, my private motto has always been that behind every silver lining, there is a cloud.[1]

I tend to follow a very nocturnal sort of existence mainly because there is something innately calming about night. Sunlight tends to depress me, in fact. & my schedule is more or less determined by the angle of light as it hits the earth – when it does, it means I am busy. Naomi Shihab Nye says she gave up the word 'busy' years ago. A matter of fact, my private reason for doing anything has always been that I'm too *busy* not to.

I

"If I were not a physicist, I would probably be a musician. I often think in music. I live my daydreams in music. I see my life in terms of music."

-Albert Einstein

INTRODUCTION

I often think how fortunate I was to have been brought up in an environment where music was always present. Who knows what would have become of me otherwise. But I have yet to come up with an answer.

--Glenn Gould

Lake Simcoe bird calls in the distance, autumn
Yellowed leaves & pines cluster about the small house.
Idealic cottage where I play with her,
Sing each black/white note hit from across warm room:

Chickering piano echoes yellow
Chickadees in trees that learned to fly
As I learned to read music, before words,
Memorize notes on spot. What minutia.

Flora's night & day phonograph playing,
Uterus singing, organ praying love
Paid off. Oh Mama, you brought me music,
taught me all you know, but what of my heart?

PROCESS (1)

skin & stretch the pig, softened,
tacked to a window or door frame,
velum hardens under suns; holed
bits grow —impurities tanned &
painted into flowers in scriptorium,
chemical bond between scribe & reader,
soot & ink.
Manuscript.

TEMPUS

Tempus suffered back problems, carried too many clocks & watches,
A letter,
& a heavy burden of knowledge.
He'd seen wars & anarchy, corruption & Constance.
Wrapped in leather, pressed to his ribbed chest,

The letter was folded in thirds. Unsealed, it might contain
Truth,
A *veritas* for a man yet to be born.
Fates decided that he would live for music & alteration.
That he would be loved but always released for genius.

Tempus held the letter for him, determined to deliver it on time.
Canada,
He'd heard, was a delightful place.
Tredging across countries was nothing for Tempus, who had lived
Quite a bit longer than you or me. He loitered in shelters,

In fields, in hollow trees. He wandered across couples naked in
Wedding rings
& wondered what mundane intrigue would break his charge.
No one could have guessed *the world*. Fate, it seems, can be cruel.

MAESTOSO[2]

What wash of impressionistic sound wave
Waits just behind his ear, waits disjointed
In polyrhythmic energy, released
In vocalized keying of ivory,
Long legs crossed —left over right—peddled foot
Tapping in double-tempoed melody?

CROSSROADS 2013

UPRIGHT

Practice rooms: large enough for upright & chair
—not *your* chair. This one: grey blue & metal, dipped seat.

Thinned walls filter violin & tuba from rooms 4 & 6
—blended Americana & Baroque beating silence into your brain.

Your fingers touch no keys, no wood, nor do they rub long black sharps
—one hand on lap & one on chin, music echoes on inner skull.

THE EAR

when gould listens, he uncurls each ear,
cartilage under fingertips & skin,
rolls upon rolls, a shaped dome,
pale & waxy.

there must have been some error,
or extra-sensorial connect, something
of the pulling in childhood, a newfound syntax,
altered & veiled.

my mother pulls my earlobes, as one might
rub temples or the bridge of the nose.
but drums in my head are ordinary,
tympanic & mortal.

II

He frightens me. I've never heard music
like this man's, this sobbing
in the midst of triumphal chords,
such ambrosial anguish,
jigs danced on shimmering coals.

-Rita Dove
"Polgreen, Sight Reading" from *Sonata Mulattica*

METHOD

My fingers are raw & cracked after six hours of continuity.
Beethoven's *Pathatique* never sounded so literal, so lethargic.
Misunderstood. Emotion & execution
Are vastly different regions of my brain
Riddled with elektraed rivalry & ribaldry. Practice,
It seems, doesn't always make perfect – especially when flailing
C-minor remains muscle memoried incorrectly.
Perhaps Gould would have thought it menial, slaving
To exactness. Perhaps I wish I had too.

OPUS I^3

String Quartet, dressed in black, glances through glasses
From corners of eyes, peripherals grasp
Each stroke: a strong vibration, a note played
On fingers, played on strings, played on sinews
Of the heart. Head shakes as the violin
Takes control & the cello echoes in lowed
Beating of battered cacophony. For,
It's all in the resolution. Still.
Disjumbled sound out of ordnance, out
Of beat, out of his head, follow their own
Singular track, each theme works against & with
The flow of blood in the veins. With final
Goodbyes, the violins each turn their leaves.

PROCESS (2)

notation & theory: the basis
for each partita, for lone string quartet.
this is the art for northern wolves,
howling notes between lines of trees,
composer & instrument. each dot or
round marking of pen exactly paced
respectively on opposite clefts, forgoing
circle of fifths

STATUS QUO

Friday afternoon, 2:47pm. I'm frisky.
Banquo & I have journeyed to the dog park where
Under light-filled trees, he joined a Pomeranian
& Labrador in a game of capture the flag. On a bench,
I relax my spine & overcoat into grained wood
Clinched by iron & bolts. Whirlies fall about me,
Green spintops: the physical manifestation of my thoughts
Falling to the ground in hard little torrents: seeds for tomorrow
Before I melt into bronzed time.

ATONAL[4]

This is a neurotic piano that thinks it's a harpsichord and I am going to close it now because it's too noisy.

-- Glenn Gould

Music is mental, not physical, the **de de te dee**;
Why practice on piano when one has a mind?
Psychoanalysis: parallel of humming & withdraw
From outside world, or harpsichordist asked
How the *you* yields to earth to play concertos in public.
Hello, this is GG here. I am a humorist. Invention: S.F. Leming,
Psychiatrist created: control negative realties in search of
Fictitious comedy. All that glitters is not Gould. Stop.

THE KEEPER

Tiptoed notes –like backstage mistress,
who smiles in love & shared secrecy.
She fingers weighted curtain which drags on stage,
knows he plays this one for her.

RHYTHM[5]

Go. gould rocks, hat half covering
turbulent mahogany locks
& antidepressant-filled brain.
perhaps this shield,
full woolen armor,
counteracts the constant
inner cantata which
practiced itself on cerebral
cortex. & gloves kept red iced
fingers from warming keys
of ivory & keys to the heart.
odd then, despite rickety seat
& vocalized performance,
layers lay abandoned dressing
room jumble next to 13 vials
half empty of blue, white, &
yellowed emotion. *Stop.*

THEME[6]

It is a travel companion, without whom I cannot work, without whom I cannot play. I have been using it for twenty-one years, this…thing – that we could also classify as a chair!

--Glenn Gould

Counted companion closer than Bach,
Cut off at legs for optional half-turnbuckle
Adjustment, is built by beloved hands.
Bert Gould adds four brackets on bridge chair,
Treasured by son who wore through wicker
With constant use. Soon, only the frame remains,
Carried folded under arm from studio to stage.

FAMILIAR

Sometimes I fill with guilt, heavy stomach
—hiding in this bar where smoke riddled chatter
keeps *him* from entering.
Gould's love is too much, bittersweet
—love which overwhelms like hoppy beer,
a form of IPA kinship.
When Glenn asked me to be his brother, legally,
my heart froze to whisper,
I already have brothers.
But let us be friends.

APPOGGIATURA[7]

Who is the boss, the soloist or the conductor? …this time the discrepancies between our views are so great that I feel I must make this small disclaimer.
--Leonard Bernstein

Unorthodox performance: Gould &
Brahms duel with D minor Concerto.

Howls of derision, the unfit public
Performer. Harold Sternberg, quite

Set on Gould's technical incapability
For playing faster. But Lenny knew

In all his genius: a great kerfuffle.
Gould: *This concert business just had to go*.

I seemed to be the only one in good
Spirits. I thought it was delightful.

PRELUDE

Hips at 90° angle are perpendicular prior to performance. As index finger touches keys, Gould relaxes vertebrae into curved ‘C,’ caressing air & audience with accentuated voice.

PIANISSIMO

Quandriness: The Question of Instrument
Remains debate of expression over
Inention, an adoption of resource.

Bach sees his first piano: deathbed
Beast – sound & timbre – the unimagined
Elements of slavework composition.

Scat jazz, ultimate violate: frequent
Improv: the 'x' of *instrument* 'x' or,
Delight of structural sound integrity

Beckons argument of dynamics &
Pressure & pedals: *pianissimo.*
Gould: indifferent to suitability,

Plays chromatic fantasia first &
Last time, conducts himself with waved hand, an
Off kilter-tenor. Debate comes to end.

III

I accept chaos, I'm not sure whether it accepts me.

-Bob Dylan

RESTITUTION & REVISION

CD318 has multi-sized strings which crisscross, overlapping gold to bronze. Felted hammers, white hammers, blue-covered hammers lift & fall – a direct cause & effect encased in hardwood. Open, sound reverberates through empty hall, but for the lone man in row five. He stares at his lover, her shine & timbre, memorizes each line & grain. Her whiteness. Her grandeur. But he will no longer touch her, will not sigh at her frame. Tempted.

TO MY MOTHER WHO IS DEAD

You slept as I wrote you then, the last letter I licked & stamped to you, as if somehow love could be transmitted through saliva. Banquo licked it too, before we handed it to the postman, a rather cheerful fellow.

You would have enjoyed his tail waving, as I must do in words –spoken, written. Even so, there is a lack of movement I never thought necessary before. Could you feel that melt through my pores, through the phone's microphone?

I thought today of our dock on the lake, how even in winter its motion retained some semblance of warmth – a short diametered peninsula. I am knocked bare & naked, coat clutched in raw palm. Flora. Ask me why.

MODULATION[8]

Dear Walter. STOP. Under the weather yesterday. STOP. X-Rays reveal chronic Bronchitis in right lung. STOP. Feeling as foggy as it is outside. STOP. Comfort from dubious doctor. STOP. It would suit you perfectly. STOP. Concerts tomorrow & Monday canceled. STOP. Cannot, *will not*, leave this room. STOP.

End.[9]

KLAVIER SONATEN

Romantic. Unsually so.
Recorded during a courtship perhaps?
You see, it is haphazard, sweet
falling notes –swells of soft theme.
Loving Gould is a dangerous pastime
Yet years after his death, I am
certain I would.

ZWISCHENSPIEL[10]

Wing folded, Alecto[11] climbs blood slick walls,
Breasts free to darkened air as Virgil leads
Pill-rattling Gould into the city
Of Dis. She moans; scaled tail rubs hip. Knotted
Hair half covers face of Cornelia Foss
Whose wedding ring dangles around long neck,
Whose voice echoes looned Muskoka[12] waters,
Whispers *dear, the mountains are looking blue*.
Blood vessels burst after two hours of chilled
Cocktail & strip searched skin: numbed for wanting.

VOCALIZE[13]

Dear Banquo ,
Thought you might like to know about the dogs here. One sees very few indeed. Most of them were killed in the war & since then it seems to be considered very bourgeois to keep a pet. The most prevalent variety is a sort of unclipped poodle-a few mongrels & no collies whatsoever. You would have the field all to yourself if you were here. You would have been able to break up a cat fight outside my window this morning. Clean up your dish like a good dog. GG[14]
--Glenn Gould

toronto, home of my birth,
remains most terrifying audience
whom i shall
never speak ill of unless, of course,
they are artists. tricky, the silent task
of stating one's own opinion, nay
purging dismal thoughts though media:
some kind of circular perpetuation of fame.

hail, stromberg.
bravo for special insensitive simpleton critique.
thankfully sir, public opinion has got the best of you
who is ruined in eyes of critics everywhere.
now, politico predisposition for enlightened conversation
in as far as provocative chatter can manage,
is far better an alternative to tainted reporters, no?
but then positively drabber is the infernal artist.

no one likes an artist
whose conversations are drearily close-minded.
they use their own imagery to such an extent that they

exclude the world from much of their point of view.
never mind, it certainly isn't worth such effort to think on.
such stratification. rather limiting, n'est-ce pas?
pen pal wanted: foreign service,
communications, dignitaries. artists need not apply.

banquo,
if only the world were just you, me & bach.
little else is necessary.
animals really are the best conversationalists.
you don't talk back, don't question my
every move & you allow me to sing
without garish interruptions of why,
or worse, *when*.

when will i return to the stage?
ludicrous. but it cannot be helped
i suppose, this circle of scavengers. it is possible
then, that this ridiculous line of questions
will end only when i am dead; what a thought.
this just needs to stop. until then,
i will sing to you, my true friend, who listens
only to sweet sounds from my head.

IV

It is cruel, you know, that music should be so beautiful. It has the beauty of loneliness of pain: of strength and freedom. The beauty of disappointment and never-satisfied love. The cruel beauty of nature and everlasting beauty of monotony.

-Benjamin Britten

THE IDEA OF NORTH

Connections are narrow up here where loneliness
Limits interpersonal communication.
What kind of being is attracted to such
Isolation?
The Eccentric, certainly, & the Writer—
Perhaps all those creative thinkers whose mind
Entertains more than glossed lips or
Vocal chords.
I have a phone line —two actually—
One wired & one which sends radio waves to towers,
That connects a small GPS chip to satellites
So I am never truly lost.
Still, your voice tethered on electric currents,
Tidal variations of digital sound complexity
(Love encoded & wrapped around my finger)
Keeps me.

NEWFOUNDLAND

npr presents glenn gould's *the latecomers,*
post-coitus program that consoles my isolation with others
the coming results this way, always disappearance into mist.
time twinges, then crawls in cadence with voices.
we have operated like this for twelve years,
hims & hers aside.

woman whispers of one car which passed by,
snow tires & chains, as she watched from behind heavy curtains
& thick panes. it paused, she noted, wondered at singular
tower on tundra. her book pressed against thighs which warmed
under pressure before it moved on, fading into snow.
perhaps tomorrow.

PROCESS (3)

stock & wound, black tape circles
between levers & dials, low bent
technician repeates & rewinds,
perfectionist's orders. studio:
soundproof room which breaks
gaze & awe of audience, where
his word is law. two things exist:
microphone & chickering.

SOLITUDE

Latecomer, you may listen
but you must allow them to take you over, the voices.
tell your stories as they have;
the arctic of your mind,
it fascinates me.

how many hours would you say,
do you need alone, for each
you spent in my company?

Cornelia?

CAPRICCIO[15]

-You're not taking this very seriously, even though I'm going to go off now to Toronto with the children and I'm marrying Glenn Gould.

-Don't be ridiculous; you're not going to marry him. I'll see you next weekend. Have fun.

--Cornelia & Lukas Foss

Uncle Glenn visits Essex House
Years later, says soft goodbyes.
Only children he'll ever know
Confuse paternal loyalty &
Man who brought collie, took
Long car trips with music high
& lake-spun Ontario musings.

I am reluctant & embarrassed
To admit that four years of love is
Gone, *forced to witness* prevailing
Absence of them, & absence of me.
Waynescott, Long Island: Final meeting.
I cannot easily surrender. Long walk
On beach brings words of futile regret.

THE ACCIDENTAL[16]

The appearance on the page of a thicket of accidental flats followed by a thicket of accidental sharps and naturals tells the eye as well as the ear of metamorphosis.
--Susan Youens[17]

He traipses in triumphed quartz not gilded
Under pressure of genius or heavy
Winter boots. The wild Atlantic can match
Magnitude, Magellan-like compass points
To inner workings of well-contempered
Claviers, can match ached soul: the Perfect
Audience, never ceasing or quenched
Of thirst. The waves clap against mangled mind
Answering in uncontrollable sound
Which cannot be altered or corrected.
Mother nature, you see, makes no mistakes.
To protect from spray (or acidic leech
Of thought), he wanders on the edge of worlds
—Black coat capsulate—daring Poseidon.

POLYRHYTHMIC[18]

But this pill complex of mine has been grossly exaggerated…One reporter wrote that I travelled with a suitcase full of pills. Actually, they barely filled a briefcase.

--Glenn Gould

Hypochondria, where blood vessels burst,
Is houred blood-pressure tests & noted
Temperature, the land where swelling or
Headache means aneurism, a statement
Of early demise: *I will not live past fifty.*

Guilted hours of remorseful discussion
Despite deathcalls over telephone wires
Which cross like synapses in the brain.
His hatred of hospitals remains even
After Flora's demise. But oh, the germs.

Oh Drs. Bennett & Andrews, or Allan who
Diagnosed separate back spasms, anxiety,
Chills in need of Valium/Ketamine cocktail.
Two-thousand pills down doctor-shopper's
Throat for nine months before death…at 50.

DOORKNOCKERS

Half-faced droop, he startles:
I'm in hiding, he says
You weren't supposed to find me.
You might as well come in,
For I am certainly not coming out.
Would you like a cup of tea?
To devour my memories?
Just read The Post.

CODA

In white blue hospital gown, sans gloves, you stare hapless
Toward ceiling, seeing without being. Your chest rises with
Machined beep, diaphragm inflating as if a rubber balloon
Blows up in your chest every six seconds, one-twenty BPM.
Fingers twitch but not in purpose, not to hit C4's white
Shined connection – hammer on golden string vibration.

FIFTY50

I sometimes wonder about the inner lives of polar bears.[19]
this slow-motion room where stale air is filtered through tubes
& travels through them, through clear plastic, before
being pumped into a ¼" slit, a transition from mechanical
to organic –keeps you. This is your personal cacoon,
your cotton woven blanket cacoon, cold like time.
skin wrinkles & bloats in this state, yellows too, but this
is just the transformation, your final metamorphosis.

ORPHEUS

Hypermobility: clear defecit
in piano play (only mulligan)
when distal pahalanx sticks in finger lock,
its knuckle pains in over extension
while mastering Hanon's trill exercise.

Left hand ring finger, nine millimeters
longer than index: sure sign of raging
testosterone. Casanova Pattern.
Apollo art: interphalangeal
joint at forty-five angle twists.

Digitus quartus, the Anamika
we've given a name (who cares for Sanskrit),
contains vena amoris —vein of love—
direct line from silver band to your heart,
If only you loved Handel over Gluck.

Sitting on an Antique Sofa in Weston, Massachusetts on a Summer's Evening

Yuri sits on mahogany bench & runs fingers
Across keyboard, tests A-minor tri-tone
Before Handel's *Overture* is hammered on strings –
Quite different from its notated pluckings.

Frowns wrinkle his forehead in concentration
& he disappears into August dusk.
Between the two of us –his fingers,
My natural inclination to hum to whatever

I might hear –Gould becomes us, & we him.
As the crickets keep our time, our natural heartbeat,
I can see him across from me. Hat low, Gould
Sits on an antique sofa, legs crossed, & smiles.

PRODIGY

The mental imagery involved with pianistic tactilia is not related to the striking of individual keys but rather to the rites of passage between notes.[20]

--Glenn Gould

I watch the next Gould who on black stage performs sonata at age five;
Carmen Sciala needs no sheets of played language – notes on lines – to tell
him how long to tap C-flat. Nor will he require pills for morning's light.
Junior only needs baby grand open to sound with hammers pounding strings.
Fine Fingerings.

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"Orpheus" appeared in *Midwest Literary Magazine*.

ENDNOTES

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[11] Glenn Gould
[1] Is used to suggest a majestic manner of performance, either in mood or speed.
[1] Composed by Glenn Gould
[1] Atonal music is music that has no specific tonality, is not in a specific key and therefore has no specific 'home' note or chord. The word atonality refers technically to various forms of 20th century music not in a key. *Naxos*.
[1] Rhythm, an essential element in music in one way or another, is the arrangement of notes according to their relative duration and relative accentuation. *Naxos*.
[1] A theme is a complete tune or melody which is of fundamental importance to a piece of music. Naxos.
[1] *It.* Support. An embellishing note or tone preceding an essential melodic note or tone and usually written as a note of smaller size. A grace note.
[1] To shift to another key.
[1] Glenn Gould, Hamburg.
[1] Music between the acts of a play or opera, entr'acte.
[1] "Alecto." *Encyclopedia Mythica* from Encyclopedia Mythica Online. Alecto is one of three Erinyes (furies) who drove their victims mad.
[1] Muskoka, Ontario
[1] A vocalize is a vocal work, whether an exercise or not, that has no words. Naxos.
[1] During Gould's 1957 Tour of Russia, he sent this postcard home to his dog, addressed: Mr. Banquo Gould, 32 Southwood Drive, Toronto.
[1] A quick, improvisational, spirited piece of music.
[1] "Accidental, adj., n., and adv.". OED Online. December 2011. Oxford University Press. Of a note: raised or lowered by one or two semitones, in momentary departure from the key signature; being or marked with a sign indicating this.
[1] Youens, Susan *Schubert's Poets and the Making of Leider*. New York: Cambridge University Press. 1999.
[1] The simultaneous combination of contrasting rhythms in music. Merriam-Webster.
[1] From "Trouble," *All-American Poem*, Matthew Dickman 2009.
[1] *Glenn Gould Reader*, "A Biography of Glenn Gould," p 445.

PARENTS' PERSPECTIVES OF SUPPORT SERVICES IN RELATION TO THEIR ANXIETY LEVEL

THROUGHOUT THEIR CHILD'S HOSPITALIZATION

RACHEL N. WERNER

ABSTRACT

The current study examined the role of support services in the perceived anxiety level of parents of hospitalized children. The study used a non-experimental, correlational survey research design. The sample was parents who stayed at Ronald McDonald House of Long Branch while their child endured inpatient or outpatient hospital treatment. It was hypothesized that support services and a positive interaction from the primary medical provide would decrease anxiety. The sample was comprised of 1 mother. Her daughter stayed with her at Ronald McDonald House while she received outpatient treatment. The subject reported a good relationship with her child's primary medical provider; he/she was gentle with her child and was available to talk on times that were convenient in the participant's schedule. She thought that Ronald McDonald House helped reduce her anxiety and helped her get through the difficult time. When she was asked about ways to improve Ronald McDonald House, the participant suggested that there be an exercise option available for the parents. Due to a lack of participants, no statistical analysis could be completed to analyze the results. As seen in the low response rate, it is very difficult for researchers to obtain a sample in this population.

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In the year 2009, there were over three million children/young adults between the ages of 1 and 21 discharged from hospitals throughout the United States (U.S. Department of Health and Human Services, 2011). Each year between the years 1996 and 2006, an average of 16,375 teenagers between the ages of 12 and 19 died (Minino, 2010). The five leading causes of death in this age group are "accidents, homicide, suicide, cancer, and heart disease." Unintentional injuries comprise about half of the deaths of teenagers. In 2007, there were 2,302 deaths of children between the ages of 0-19 caused by neoplasms (Selected Causes of Death, 2007).

All of these statistics support the statement that children can be brought to the hospital for a variety of reasons. In 2009, respiratory system problems such as asthma and pneumonia were the leading causes of hospitalization in children ages 1-9 (U.S. Department of Health and Human Services, 2011). The most common cause of hospitalization of children ages 10-14 were mental disorders and the most common reason for hospitalization of

adolescents and young adult females ages 15-21 was labor and delivery. Around 1,000 children are diagnosed with End-Stage Renal Disease (ESRD) each year (Silverstein, 2004). All of these statistics express the sheer number of children who are treated at hospitals throughout the world, yearly.

Hospitalization can be an extremely stressful experience for anyone, including children. Salmela, Aronen and Salanterä (2011) interviewed children ages 4 to 6 and concluded that for children in this age group hospitalization might be stressful enough that it could be damaging to their well-being. The hospital is somewhere where health problems should be treated, not caused. Aley (2002) found that when children are put in the position of experiencing hospital-related fears, it could be traumatic enough to have a negative effect on normal development. It is possible that something as trivial as the color of the nurse's clothes could have an effect on the children while they are hospitalized (Roohafza, Pirnia, Sadeghi, Toghianifar, Talaei, & Ashrafi, 2009). The researchers concluded that when nurses wore white outfits, instead of colorful ones, children reported significantly higher anxiety levels. This research highlights the susceptibility that children have for anxiety while hospitalized and the need for many different aspects of hospitalization to be considered when looking at children and anxiety.

The hospitalization of a child not only has a large impact on the child, but the parents as well (Aguilar-Vafaie, 2008; Alexander, White and Powell, 1986; Bronner, Kayser, Knoester, Bos, Last and Groostenhuis, 2009; LaMontagne, Hepworth, Salisbury and Riley, 2003; Needle, O'Riordan, Smith, 2009; Wolfer and Visintainer, 1975). Parental anxiety is a very common result of a child's hospitalization (Aguilar-Vafaie, 2008; Alexander, White and Powell, 1986; Bronner, Kayser, Knoester, Bos, Last and Groostenhuis, 2009; LaMontagne, Hepworth, Salisbury and Riley, 2003; Needle, O'Riordan, Smith, 2009; Wolfer and Visintainer, 1975). Keeping a child in a hospital at low anxiety levels can be very difficult if the parent is experiencing high anxiety levels (Melnyk, 1994). Many different aspects of the child's hospitalization can contribute to the anxiety of the parents (Castillo & Vilchez-Lara, 2011). The anxiety that a parent feels when their child is in the hospital could in turn have an effect on the child's anxiety (Aguilar-Vafaie, 2008; Melnyk, 1994).

It has been found that parental involvement in a child's hospitalization has a significant effect on the quality of life that the family leads (Wysocki & Gavin, 2006). It is important for everyone in the family

that the parents are present during the child's hospitalization. There have been support systems created to provide the necessary comfort and therapy for ill children and their parents as well (Burke, Harrison, Kauffmann, & Wong, 2001; Ellerton & Merriam ,1994; Mather & Glasrud, 1981; Melnyk et al. 2004; Melnyk, Crean, Feinstein, Fairbanks, Alpert-Gillis, 2007; Stratton, 2004; Wolfer & Visintainer, 1975). These support services can range from education about their medical situations for the patients and their families to computer programs provided that connect children with other kids in the world that are going through similar situations (Boukydis, 2000; Cashin & Witt, 2010; Child Life Services, 2000; Commitee on School Health, 2000).

The purpose of the current study is to examine the perceived effect that support services offered in hospitals have on parents of children who have endured a hospital stay. Parents of ill or injured children are extremely vulnerable to poor mental health in the areas of stress/anxiety. Bronner, Kayser, Knoester, Bos, Last & Groostenhuis (2009) conducted a study on parents of children who were unexpectedly hospitalized and found that at a three month follow up, 23.4% of the 128 parents involved in the study reported potentially clinically significant levels of anxiety. The mental health of the parent can have an effect on the mental health of the sick child (Astin, 1977). It has been found that a change in emotions can cause a change in immune responses (Matsunaga et al., 2008). The results of this research highlight the necessity for parents to be at the highest level of functioning of which they are capable. It is important to provide parents with every service possible that can help reduce emotional and mental distress. The current research will help determine the triggers for anxiety in the parents of children who were patients in a pediatric ward. It will also help determine which support services are related to lower levels of anxiety. This research may also help therapists look into new ways of helping people cope with their child's illness and in turn, experience lower levels of anxiety.

Causes of Parental Anxiety

Parents who have children in the hospital are emotionally and mentally vulnerable because of their current situation, which makes them more susceptible to mental health problems such as anxiety (Aguilar-Vafaie, 2008; Bronner, Kayser, Knoester, Bos, Last & Groostenhuis, 2009; Needle, O'Riordan, Smith, 2009). Causes of anxiety among parents of hospitalized children can be related to: family related stressors, hospital related stressors, or stressors that result directly from their child being ill.

Family related stressors can come from many different aspects of family life; examples may be, having other children at home, the travel time to get to and from the hospital, and their financial situation (Alexander, White & Powell, 1986; Latva, Lehtonen, Salmelin & Tamminen, 2007). Latva, Lehtonen, Salmelin and Tamminen (2007) surveyed parents of 210 infants in the neonatal intensive care unit (NICU) of Tampere University Hospital and found that parents visited their child in the NICU less frequently if they had other children at home and if the travel distance from home was more than 30 kilometers. Alexander, White and Powell (1986) found that parents who have more children at home reported higher anxiety. This could be due to the extra stressors that having more children and responsibilities could cause, such as alternative care for the child, providing meals for the children at home, and coordinating activities in which those children might participate. The extra time that the parents are required to spend at home could add risk factors for anxiety related to their child that is in the NICU.

Along with family related stressors, hospital related stressors are a common cause of anxiety in parents of hospitalized children. Parents of children who were treated in a Pediatric Intensive Care Unit (PICU) had what qualified as high anxiety when it was assessed within 24 hours of the hospitalization of their child (Needle, O'Riordan, Smith, 2009). A major stressor that occurs during hospitalization is medical procedures that occur throughout the time of hospitalization. Piira, Sugiura, Champion, Donnelly and Cole (2005) studied parental presence during their children's medical procedures and the effect on the child and the parent. Although there are no decreases in child distress when parents are present during medical procedures, there are potential advantages to parents (Piira, Sugiura, Champion, Donnelly & Cole, 2005). Parents who were present during medical procedures reported less distress and more satisfaction. This suggests that medical procedures on children are stress-causing experiences for their parents.

Aside from what takes place while the child is at the hospital, a common stressor for parents is the sole fact that they have a child that is in the hospital. When a child becomes unexpectedly ill, it can have a direct impact on the anxiety of their caretaker. Bronner, Kayser, Knoester, Bos, Last and Groostenhuis (2009) conducted a study on 149 parents whose children had become unexpectedly ill and found that a significant amount of parents had mental health issues such as PTSD (10%), anxiety (23%) and

depression (15%). Mental health problems such as anxiety display themselves differently in every case. What mental health professionals can do to reduce this problem, is produce effective prevention and interventions programs.

Theory

Research has concluded that parental anxiety is affected by having a child in the hospital. Since anxiety stems from different stressors for everyone, it is important to cover all of the stressors when devising programs to reduce the anxiety that parents experience. To provide successful devices for the parents to feel more comfortable, it is necessary to assess different stressors and find ways that health care professionals can attack of each of them.

People in different situations rely on different strategies to cope emotionally and mentally (Goldbeck, 2001; Snethen, Broome, Kelber & Warady, 2004; Martin, Calabrese, Wolters, Walker, Warren & Hazra, 2012). Snethen, Broome, Kelber and Warady (2004) examined coping strategies that teenagers with End Stage Renal Disease (ESRD) used to cope with their chronic illness. The researchers compared the responses from participants with data that was previously published by (McCubbin et al., 1996). When compared with coping strategies of healthy teenagers, the chronically ill adolescents were found to have significantly different scores on 9 out of 12 subscales used in the study. These nine subscales were: venting feelings, seeking diversion, developing self-reliance and optimism, solving family problems, avoiding problems, investing in close friends, seeking professional support, engaging in demanding activity, and relaxing. Of those nine subscales that were significantly different between the two groups of adolescents, the adolescents with ESRD utilized avoiding problems and venting feelings significantly more than well adolescents. Although they used different strategies to cope with their illness, the adolescents with ESRD did not have an overall different coping score than well adolescents. This suggests that providing support for ill children could be more successful when using different strategies than those used for healthy children (children without a major injury or serious illness). It is possible that this is the case with parents as well. If ill children use different strategies to cope than well children, it is highly possible that parents of ill children use different strategies to cope than parents in different situations.

Parents of children with cancer use social support coping strategies, searching for support from people around them, family and non-family (Martin, Calabrese, Wolters, Walker, Warren & Hazra, 2012). Goldbeck (2001) studied parents of children being treated at a pediatric oncology clinic within three months of diagnosis, in contrast to a control group made up of parents of children with diabetes and epilepsy (this control group accounted for any disease-specific effects that cancer might have on the coping of parents). Coping strategies were examined along with parental quality of life and child quality of life. The analysis of the data was a 2X2 factorial design examining the effects of gender and diagnosis of the child on the coping style and quality of life. Parents in the oncology group reported a significantly lower quality of life than the parents in the control group. Mothers leaned on social support more than fathers. Mothers were also better at maintaining family connection, had more optimism, and had better understanding of their child's medical situation. Fathers in the oncology group did not use any of the coping strategies more than any of the other three parent groups (control mothers, control fathers and oncology mothers). This research highlights the necessity for parents to be provided with different services based on what helps them the most. It is important for the parents to get the specific help that they need.

The theory that support services will reduce anxiety in parents of hospitalized children, will only truly be successful if the right prevention/intervention strategies are used. It is necessary to develop effective interventions that can be used for parents who are at risk for experiencing high levels of anxiety. There are many different systems that have been developed to help parents through the process of having an ill child or a child in the hospital which can then in turn reduce parental anxiety. Some of these services have been examined in relation to their effects on the children and their parents. The preventions and interventions have been found to reduce anxiety, make the hospital stay more comfortable and enjoyable and improve family functioning after the hospital stay (Burke, Harrison, Kauffmann, & Wong, 2001; Cashin & Witt, 2010; Melnyk et al. 2004; Wolfer & Visintainer, 1975; Stratton, 2004).

Prevention/Intervention

The health care professionals that parents come in contact with, while their child is in the hospital, can make an enormous impact on the anxiety that parents feel. Horn, Feldman and Ploof (1995) found that parents

who had children that were enduring an extended stay in the hospital depended on being provided with honest and complete information and getting support from health care professionals. Stratton (2004) interviewed parents of children who were hospitalized for: croup, respiratory syncytial virus, meningitis, dehydration, mild trauma and spinal surgery. After coding and analyzing the qualitative data it was found that the relationship between the child's medical caregiver and parent and child are statistically significant to the process of healing. This relationship is the base layer of the medical caregiver meeting the needs of the parent and child. In order for these needs to be met and the relationship between the medical caregiver and the parent and child, the parents must be present at the hospital. Once the guardian of the child is able to develop a relationship with the medical provider, the needs of the child and the parent will both be met more adequately by the support they receive. The thought that family's psychological needs should be of high concern in these situations was brought to light by the field of Child Life (Wyles, 2004).

Child Life Services is a department in most pediatric wards that is a setting for health care professionals who offer support services in many different forms to children and their parents in the hospital. The Child Life Council was created in 1982 and established professional certification in 1986. Before Child Life was created, children were not given explanations for what they were doing in the hospital and what was being done to them. They reacted poorly to hospitalization and could have experienced long term anxiety and changes in development from the terrifying experience. Now, it is the job of Child Life Specialists to prepare children for operations and procedures, explain all "scary things" in kid-friendly terms, and help children cope with anything related to their hospital stay. Some of the services that Child Life uses to help children and their parents through the hospitalization process are: play experiences and therapy, psychological preparation for surgery and other procedures, and education for families to promote family support (Child Life Services, 2000). Some Child Life Services programs also offer educational or school programs in the hospital (Mather & Glasrud, 1981). An on-staff teacher at The Department of Pediatric Hematology/ Oncology of the University Hospital of Heraklion found that children really wanted to attend school and reacted well to an educational program that incorporated play therapy (Kapelaki, Fovakis, Dimitriou, Perdikogianni, Stiakaki & Kalmanti, 2003).

One of the programs that is run by Child Life Specialists is the Starbright World Program (Cashin & Witt, 2010). The Starbright World Program was developed by the Starbright Foundation, and is a program developed to help children learn how to cope with being in the hospital. The program utilizes technology to: educate the children on their illness and upcoming procedures, allow the children a form of communication to other children who are going through similar health problems and entertain the children with video games and activities that are creativity related. A major aspect of this program is the connection it allows children to have with others who are experiencing the same scary situations. It gives them the opportunity to have a buddy who could be halfway around the world, someone they can talk to through video chat, share jokes and websites with, and play games against. When studied, the program received overall positive reviews from patients and families. Children who were undergoing hemodialysis treatments for ESRD reported that the Starbright World Foundation technology allowed them a channel for distractions of activities and games (Sanderson & Barry, 2003). It also allowed them to develop friendships with children undergoing similar medical treatments. A program such as this will make the hospital stay more enjoyable and relatable to a child therefore in turn help the parents. Although there is research on the Starbright World Program, there is no research that highlights whether this program reduces anxiety of the patient and the parents. It is imperative that this is studied because the care and support that is provided for the child can provide different risk factors for parental anxiety.

Another program that most Child Life Services offer is a pre-surgical operating room tour and psychological preparation for surgery for parents and children. The purpose of this program is to desensitize the child and their family to the environment of the hospital including the operating room. It was designed to reduce stress and anxiety once the time for the procedure comes. The effectiveness of a similar program was examined by Ellerton and Merriam (1994). The program took place within a week before the child was scheduled for surgery and included a tour of the operating room, the opportunity to try on a hospital gown, practicing having blood pressure, weight and pulses taken, the chance to see nurses in their masks and boots, and other procedures that would take place on the day of surgery. The parents got a description of the side effects the child might experience so they could help the child prepare for them and deal with them. This program

received positive feedback and the majority of the parents that attended the program reported that it was very helpful in providing a more comfortable idea of the experience for the parent or the child. More than 80 percent of the parents reported that the information involved in the program was helpful for them and their children.

Stress Point Intervention is another program that nurses can use to help families cope with having a child in the hospital (Burke, Harrison, Kauffmann, & Wong, 2001; Wolfer & Visintainer, 1975). This program that is put into effect by nurses, is centered around providing the families of hospitalized children with more information and addressing their concerns. Burke et al. (2001) found a significant improvement in family functioning three months after their child's discharge from the hospital compared to before hospitalization. Burke, Handley-Derry, Costello, Kauffmann, and Dillon (1997) concluded that improvements in family functioning appear to contribute in improving the child's medical outcomes.

Having a child with health problems adds all sorts of extra aspects to parenting (Cline & Greene, 2007). It is very important for parents to have the information if, for example the child refuses to do their necessary treatments or they are not feeling well and are having anxiety about returning to the doctors. The results of all of this research highlights the necessity for parents to be near their children while the child is hospitalized, that way the parent can be at the highest level of functioning of which they are capable, even when they are dealing with very difficult situations.

Family support is exactly the field to which Ronald McDonald House Charities caters. Ronald McDonald House Charities pride themselves on providing a breath of fresh air for children and families going through difficult times. Ronald McDonald house is a place where these families can stay for as long as they need and have a comfortable bed to sleep and a warm meal for them to eat, all within a short distance to their child's hospital room (Ronald McDonald). The vision of Ronald McDonald House Charities is "We believe that when you change a child's life, you change a family's, which can change a community, and ultimately the world. We strive to be part of that change and part of the solution in improving the lives of children and their families by providing the programs that strengthen families during their most difficult and challenging times" (Ronald McDonald). They

provide the opportunity for children to always have someone by their side during any form of medical treatment. With help from the medical community, Ronald McDonald House Charities is making leaps and bounds towards making sure children get the treatment that they need and deserve and that no family ceases seeking better treatment because they cannot afford a place to stay near their child's heath care provider. If the best doctor for the child is thousands of miles from their home, the family should be able to bring their child to the best care possible. The families should not have to worry that they would have to pay for a hotel. Although Ronald McDonald House Charities are widely recognized and used, there is no research that compares the services that the Ronald McDonald House Charities offer with anxiety of the child or families.

In some cases, the programs that have been implemented have later been found not to reduce parental and child anxiety. Although Ellerton and Merriam (1994) received positive feedback from parents concerning their pre-operation, operation room tour, there was no significant difference in parental anxiety of the group that received the program and the non-intervention group in their study. There are programs that are being put into effect but are not completing the task that they were originally set out to do. The current research will examine these downfalls as well as those services that are successful in reducing perceived parental anxiety.

The Present Study

Research has examined heightened anxiety levels in parents of hospitalized children (Aguilar-Vafaie, 2008; Alexander, White and Powell, 1986; Bronner, Kayser, Knoester, Bos, Last and Groostenhuis, 2009; LaMontagne, Hepworth, Salisbury and Riley, 2003; Needle, O'Riordan, Smith, 2009; Wolfer and Visintainer, 1975). When a parent has higher levels of anxiety it could then have an effect on their ill child's anxiety (Aguilar-Vafaie, 2008; Melnyk, 1994). There have been aspects of life that have been found to be protective factors from anxiety as well (Castillo & Vilchez-Lara, 2011). Castillo and Vilchez-Lara (2011) conducted a study to examine different aspects of life and the relationship they have with anxiety. The study was conducted in southern Spain and the participants were 191 parents of hospitalized children. Some of the factors that they considered were whether the parents were immigrants to Spain or not, and family adaptability, functioning and cohesion. The researchers concluded that families with lower levels of family functioning and family cohesion displayed higher anxiety

levels than those with higher levels of family functioning and family cohesion. The researchers also found that the immigrant group reported higher levels of anxiety than the non-immigrant group. Every family has different factors that contribute to their life. This research proposes that different people might use some of these factors as a buffer between themselves and anxiety. Aguilar-Vafaie (2008) studied what parents of children being treated for cancer used to cope. The researcher found that in some situations fathers used different coping than mothers did (and vice versa). Fathers used maintaining social support, self-esteem and psychological stability significantly more than mothers did. Mothers utilized religious coping significantly more than fathers.

This suggests that different support services might assist different people in different ways. Where some support services will provide relief to some people, they might only add to anxiety for other people. There have been many support services researched that have been offered to hospitalized children and their families, but not all of them have been compared with anxiety (Boukydis, 2000; Burke, Harrison, Kauffmann, & Wong, 2001; Cashin & Witt, 2010; Child Life Services, 2000; Ellerton & Merriam ,1994; Home, Hospital, and Other Non-School-based Instruction for Children and Adolescents Who Are Medically Unable to Attend School, 2000 Mather & Glasrud, 1981; Melnyk et al. 2004; Melnyk, Crean, Feinstein, Fairbanks, Alpert-Gillis, 2007; Stratton, 2004; Wolfer & Visintainer, 1975).

Although there has been research on anxiety, hospitalization of children and support services, there are some significant gaps in the research. There are gaps in the research regarding finances that are involved with a hospital stay. Research has failed to look at how anxiety is affected by financial troubles that are a result of a hospitalization in the family. An inpatient hospitalization changes a lot of aspects of daily lives, all of which could affect financial responsibilities. An important role that parents play is the provider, and if the parents cannot provide for their children it might cause a change in anxiety that the parent experiences.

There has been research on anxiety, but there is no research that looks into how gender roles affect anxiety. It is possible that mothers and fathers experience anxiety differently as a result of their child's hospitalization. The majority of the studies that include both mothers and fathers do not allow both parents to complete the study. For support programs, there are many case studies for one of the programs that a specific hospital offers, instead of assessing all of the services.

The current topic examines further into the role that the support services play in the hospital stay as perceived by the parents. This study combines two important and heavily researched topics in the field of medical science: anxiety and services offered to families of hospitalized children. The most important question for the role of the support services is whether they are relieving anxiety and making the hospital stay less anxiety-ridden. Parents should be provided with services that will intentionally lower their anxiety and create a more comfortable experience for them. This research can also help therapists look into new ways of helping people cope with their child's illness and diminish levels of anxiety. This study will help determine the risk factors for anxiety in parents whose children were recently hospitalized. In addition, it will evaluate which support services the parents perceived to be the most helpful in reducing that anxiety. It is hypothesized that many of the support services offered will decrease anxiety. It is also hypothesized that positive interaction from the doctors and nurses will decrease anxiety. Mothers are expected to have higher anxiety levels and be more easily influenced by the support services than fathers.

Method

Sampling

A non-probability convenience sample was used to select participants for the study. Participants were parents who stayed at Ronald McDonald House of Long Branch while their child received treatment at an area hospital. The Ronald McDonald House database of families who stayed at the House in the last year was used to gain access to participants. The House Manager of Ronald McDonald House contacted the participants by email and gave them brief information about the study. She also provided a link to the study that the participants could click on to participate in the research.

Participants

The current study included a sample of 1 mother who spent time at Ronald McDonald House of Long Branch while her child endured outpatient hospital treatment. She was married and employed full-time. The only requirement for participation was that the participant must be 18 years of age or older.

Materials

This current study utilized a self-report survey created by the researchers (see attached) that was broken into five domains: parental

demographics, child demographics, hospital stay, support services and anxiety. The survey created by the researchers was not tested for reliability but it has face validity because it appears to measure what it was intended to measure.

Parental demographics domain. The demographics survey was a questionnaire that asked participants about their ethnicity, religious affiliation, age, gender, financial situation and family structure. The survey contained both moderating and mediating variables. Some of the moderating variables were gender, race, and age. Some of the mediating variables in the survey were employment situation, income and religious affiliation.

Child demographics domain. This demographics survey was a questionnaire that asked participants about their hospitalized child's gender, child's family structure, age and reason for hospitalization. The survey consisted of four questions and has both moderating and mediating variables. The moderating variables were gender and age. A mediating variable was family structure.

Hospital stay domain. This survey asked the parents about the parameters of their child's stay at the hospital and the satisfaction they had with medical providers that took care of them while they were there. Examples of the questions are "how many days was your child's inpatient stay?" and "The hospital staff was attentive". The questionnaire had many different scales involved. For the statements that were asked to be rated, the scale (1=strongly disagree, 7=strongly agree) was used. There were also yes, no questions that were used and open-ended questions. An example of a yes, no question is "Did you hire private nurses to help take care of your child while at the hospital?" An example of an open ended question used in the survey "If you stayed the night at the hospital, were any amenities provided for you? If yes, what were the amenities?" This section of the survey consisted of 25 questions and has both mediating and moderating variables. The moderator variables included how long the trip to the hospital was and the number of hours a day someone was visiting their child.

Support services domain. This questionnaire contained questions about the services that the parents and children were provided with while they were in the hospital. The independent variable of the study was support services. This survey intended to measure the positives and negatives of the support services that the parents were offered while their child was in the hospital. These questions were in three different formats: yes or no questions, open-ended questions, and Likert style questions. An example of the yes or

no questions was "Did your child participate in group therapy of any kind with other children who are in similar situations?" An example of an open-ended question in the scale was "What services was your child offered? Please list all." The Likert style questions were on the same scale that was used in the hospital stay (1=strongly disagree, 7=strongly agree) and an example was, "The playrooms at the hospital had activities for children of all ages." This portion of the survey consisted of 22 questions and has moderating and mediating variables. An example of a moderator variable in this section is the support services that the parents were offered. An example of the mediating variables in the survey is if the child made friends while they were in the hospital and if the child participated in group therapy at all.

Anxiety domain. The anxiety survey asked questions to see what the participants relied on during a high anxiety time in their lives. The statements from the GAD-7 (Spitzer, Kroenke, Williams, Lowe, 2006) were used but the scale on which the statements were rated was adapted to fit the needs of the current research. These were adapted because the GAD-7 was created to measure anxiety within the last two weeks. The researchers needed to change this because they needed to assess anxiety from more than two weeks prior.

Research Design

A non-experimental, correlational survey research design was used to examine parental anxiety in parents of hospitalized children and the effect of support services on their anxiety level. The study contained a self-report online survey. The survey was designed by the researchers and was broken down into five sections: demographics, child demographics, hospital stay, support services and anxiety. The anxiety portion of the survey was adapted from the GAD-7.

Procedure

The potential participants were sent an e-mail from the House Manager with information about the study and asked them if they were willing to participate. They were ensured that they were not required to participate. The e-mail that the House Manager sent included a link where participants could access the survey. The first page of the link was a consent form. This form explained the intention of the study and explained that all of the information they provided was completely confidential because their name were not on the survey. The consent form also stressed the right that the participants were able to leave the study at any time without penalization or question. The bottom of the consent form had two options "I agree to participate" and "I do not agree to participate". If the participant chose that

the "I agree to participate" option, the survey would then open for them. If the participant chose to participate, after they completed the survey portion, they moved on to the debriefing. The debriefing reiterated the reason for the study and gave them options of numbers to call if they felt any discomfort from the study. They clicked submit when they were done reading the debriefing. If the participant chose the "I do not agree to participate" option, the participant was taken to the debriefing page. The time commitment that was expected from the participants was 20 to 45 minutes, but they were given as much time as they needed.

Results

The sample was comprised of 1 subject. The participant did not complete the entire survey but the questions that were answered can be discussed, however no statistical analysis can be completed.

Parental demographics domain

The one participant was a mother who was employed full-time, married, and Caucasian. She had two children under the age of 13 and was not the primary financial provider for her household.

Child demographics domain

The participant's 6 year old daughter was enduring outpatient hospital treatment at the time that they were staying at Ronald McDonald House of Long Branch. The child grew up in a dual-parent household and her hospitalization was not a result of a genetic disorder.

Hospital stay domain

The participant recorded that she and her child stayed at Ronald McDonald House for 30 days. The participant rated that she strongly agreed to the statements: "Your child's primary medical provider listened to what you said" and "Your child's primary medical provider was gentle with your child." When asked if her child's care plan was devised out her family situation (i.e. Financial situation, home life and work hours) she responded yes. The participant also reported that her child had never been a patient at a hospital before the current treatment.

Support services domain

The participant explained that she felt that her child's primary care provider was available to talk to her about her child's condition on times that were convenient in her schedule. The participant felt as though being able to use services at Ronald McDonald House reduced her anxiety. She felt as though Ronald McDonald House helped her get through the time to the best of their abilities. When the participant was asked about the services she received while at Ronald McDonald House, she listed, "housing, food, social interaction, play area, internet access, gifts, birthday celebration, diapers and personal care items, hotel room in New York City for a two day trip out as a family." When the participant was asked what else could be done by Ronald McDonald House, she answered, "the only improvement I can think of is access to a nearby gym with childcare or maybe a treadmill or elliptical for a workout option in the winter. Exercise helps reduce stress, so encouraging it could improve the health of families going through difficult situations."

Anxiety domain

The last section of the survey was the anxiety section that was adapted from the GAD-7 (Spitzer, Kroenke, Williams, Lowe, 2006). On 5 of the 7 anxiety questions the participant reported that she sometimes felt nervous, anxious or on edge, thought she was worrying too much about different things, had problems relaxing, felt restless or unable to sit still and felt irritable or easily annoyed. The other 2 statements she rated that she seldom was unable to stop worrying and felt afraid that something awful might happen.

Incomplete Questions

The participant only completed about one half of the survey. Some of the questions on the survey did not apply to her situation, but some of the questions did apply to her and she chose not to answer. She answered all of the questions in the parental demographic section and all of the questions in the child demographic section. In the hospital stay section, there were questions that only applied to inpatient hospital treatments and not outpatient treatments such as the one the patients' child was enduring. The questions that did not apply to her were ones such as, "how many days was your child's hospital stay" and "was your child in their own room for the duration of their hospital stay?" She did however check that her child's hospital stay was not unexpected. The next section of the survey was about the hospital staff. The participant did not answer any of these questions. These questions could have applied to the participant because her child did interact with hospital staff

while receiving treatments. These statements were about how the hospital staff treated the parent and their child. The next section was also about the child's medical providers and hospital staff and the participant did choose two of these to answer. Some of the questions that the participant did not answer were "your child's primary medical provider listened to what your child said" and "the hospital staff explained to you the possible implication of your child's illness or injury."

The next section of the survey was the Support Services section. Many of the questions in this section were asking about services provided by the hospital and it is possible that the participant never encountered these situations. Many of the questions in this section went unanswered. The participant did answer the open-ended questions about the services provided by Ronald McDonald House.

Discussion

The present study examined the anxiety levels of parents of hospitalized children and the perceived effects of the support services that they were offered. It was hypothesized that support services offered would decrease anxiety. It was also hypothesized that a positive interaction between the doctors/nurses and parents would decrease parental anxiety. Mothers were expected to have higher anxiety levels and be more easily influenced by the support services that they were offered than fathers. The purpose of the study was to assess which support services were successful in reducing the perceived anxiety level of parents of hospitalized children. A cross-sectional survey research design was used. The researchers worked with Ronald McDonald House of Long Branch to obtain a non-probability convenience sample of parents who had used their services. The researchers devised a survey to assess factors pertinent to the study such as: what services the parents were offered, how the parents were treated by the hospital staff, how the parent's children were treated by the hospital staff, demographics and anxiety levels.

The study yielded one participant. The participant was a mother who had a six year old daughter that was receiving outpatient hospital treatment. Her daughter stayed with her at Ronald McDonald House while the she was receiving outpatient treatment. The participant felt as though her daughters'

primary medical provider listened to what she said and was gentle with her child. The medical provider also made himself/herself available to talk about the participants' child on times that were convenient in her schedule. She also felt as though her daughters care plan was made specific for their family's situation. She thought that Ronald McDonald House helped reduce her anxiety and helped her get through the difficult time.

Prior research suggests an improvement in family functioning contributes to the improvement in the medical outcome of the child (Burke, Handley-Derry, Costello, Kauffmann, and Dillon, 1997). According to the participant, Ronald McDonald House took extreme measures to make sure this family was functioning at an optimal level. The organization not only payed attention to the families immediate needs but also provided special accommodations that consisted of but were not limited to, birthday celebrations and a family trip to New York City. It is possible that these services could have improved family functioning at the time and assisted in contributing to improvement in the child's medical outcomes; however, in this study that data was not collected.

Goldbeck (2001) found that there was a difference in parental coping based on gender, with mothers leaning on social support and having more optimism more than fathers. In the present study, there was no data with whom to compare the one mother, but the participant did express that she was provided with social interaction while at Ronald McDonald House. It is possible that social interaction was a more significant support service for the mother than it may have been if a father was answering the survey, however, the father did not provide data for the researchers to examine. It is also possible that the mother was optimistic and that may have contributed to her low perceived anxiety level. It is not known by the researchers if the father was present at Ronald McDonald House with the mother and their child.

Implications and Interpretation

Although the study did not have enough participants to analyze the data for significance, the participant that did complete the survey gave the researchers some data to explore. The participant reported positive interactions with her child's primary medical provider. She disclosed that she was not happy with her health insurance. Overall, the participant reported

low perceived anxiety throughout the course of her child's hospitalization. It is possible that this low anxiety level could be related to the severity of the illness however the researchers do not have data to support this theory.

Research has shown that support from health care professionals and a good relationship with the medical care provider are important to both the ill child and their parents (Hornman, Feldman & Ploof, 1995; Stratton, 2004). The participant stated that she was comfortable with the child's primary medical provider, that he/she was gentle, listened to what she said and available to talk on convenient times. The level of comfort that the participant felt with her child's medical provider was not due to the number of times that her child was his/her patient because the subject reported that her child had never been a hospital patient before. This good relationship that the participant had with her child's doctor is consistent with the research saying that these aspects of health care are important to the process of healing (Stratton, 2004). It is possible that this relationship between the medical care provider and the mother was a contributing factor in the reason why the participant recorded such low anxiety levels.

The participant recorded low anxiety levels and a positive experience at Ronald McDonald House; however, she did make a suggestion for improvement. When asked what else could be done at Ronald McDonald House in terms of support services, she explained that she felt it was important to have an exercise option available for the parents. Whether it be access to a gym in the area, or an in house option, she felt that it would be a good outlet for stress reduction. Kim et al. (2012) examined physical activity and mental health and concluded that people who exercise for their optimal number of hours per week are more likely to experience better mental health. This research suggests providing an exercise option for the parents that stay at Ronald McDonald House could be beneficial to their mental health.

Other than this one suggestion, her experience at Ronald McDonald House seemed to be overall successful and positive with many support services offered. Despite the fact that the study did not yield enough results to support the hypotheses, the results that were provided by the one participant can be used for the improvement of Ronald McDonald House.

Strengths and Limitations

All research studies have some strengths that can be passed on for future research. A major strength of the current study was the measure. The first part of the measure was devised by the researchers, therefore explored all of the variables that the researchers felt were necessary to fill the identified gaps in the literature. The second part of the measure was a standardized anxiety measure. One of the goals of the study was to compare mothers and fathers on their perceived anxiety levels and the support services that they felt were effective.

A strength of the current study was that the researchers assessed more than one support service in the study. Most of the prior literature on support services offered in hospitals examined one support service at a time. This could be a complete misjudgment of the support services because there are multiple support services offered to families who are going through difficult times. It is likely that the support services work together to assist the families in these situations, assessing one at a time might produce a skewed result. The current study hoped to find out about the collaboration of the support services that were producing the desired result.

Another major strength of the study was assessing the support services at Ronald McDonald House along with support services provided by the hospital. This was an opportunity for the researchers to explore another important facet of the families' experience of hospital treatment. Ronald McDonald House is designed to provide assistance to families who need a helping hand, and the organization offers many different support services that parents would not get if they were not involved with the House. Ronald McDonald provides a home away from home for people who need to travel to get their child the right medical care. This is also a topic that has not been explored by literature. The research aimed to fill many gaps in the literature about families of hospitalized children. Research such as this is very important to the children's hospital and pediatric community.

As with all research studies there were limitations that weakened the study. The main limitation in the study was the lack of participants. Fortunately, with one participant, the researchers were still able to find some usable data, but there was no possibility for generalizability. There is no way to apply the results provided by one participant to the whole population of parents with hospitalized children. The researchers were expecting more participants and the opportunity to analyze data from a larger sample.

Although the measure of the study was a strength, once the study was concluded, the researchers discovered other variables that could play a role in anxiety level and support services offered. The researchers did not ask specifically if the whole family was residing at Ronald McDonald House. It is unclear to the researchers if the father and sibling were present at Ronald McDonald House with the mother and ill child. If they were not present, it could be for a variety of reasons. One of these reasons could be location and travel. It is unknown by the researchers how far Ronald McDonald House is from the participants' home. It would have been beneficial for the researchers to have asked where the location of the participants' home was and how much travel they had done to arrive at the House. In some cases, children must travel very far distances in order to get treated from a specialist that caters to their condition. In those cases, it is possible that some members of the family must stay home and resume daily life (i.e. work, school, extra-curricular activities). This could factor into family functioning, support services as well as anxiety that the participant is experiencing.

Another possible limitation of the present study could be the methods. The survey website reported that 31 people opened the link to the survey, but never opened the survey past the informed consent. An explanation for this could be that they are too busy to sit at the computer for twenty minutes to complete the study. Another explanation could be that they are accustomed to talking about their child's illness or injury in person and would be out of their comfort zone by filling the survey out and not knowing who was going to be assessing their information. The present study might have been more effective at gaining participants if the data collection was more qualitative in nature and was conducted via a face-to-face interview. It is possible that this population would react better to person-to-person interaction.

Another limitation with the methods could be that the measure was slightly confusing. The one participant did not complete the whole survey, she skipped sections of the survey that she felt did not apply to her. Some of those sections had words such as "hospital" and "hospital staff." Because the participant's child was treated outpatient, she might have felt that she did not need to answer these questions. But really, her child was still in the hospital for treatments and almost all of the questions did apply to her and her child.

If the questions were reworded to explain the instances which they were asking about, it is possible that she would have completed the whole survey.

Another limitation is that the researchers were not aware of the current situations of the participants and their children. Since this was an online survey and sent to past residents of Ronald McDonald House, it is possible that the participants' children are still ill or having medical troubles. It is also possible they are having new medical issues than they were previously and the parent is too anxious to talk about the current health of the child. In this case the potential participants might just be too busy with their lives and caring for their children to read their e-mails or did not have the time to sit for twenty minutes to complete the survey. In that case, they might have experienced different situations that could have affected the survey.

Present Study Process

Due to constraints of time, access to participants and ethical obligations, the present study had to be modified many times. The process of conducting the study was very different than what originally was desired by the researchers. This difficulty of the process could be of one the reasons that there is a lack of research on the population. It is evident from the low response rate that this population is very difficult to reach. Part of this problem stems from ethical concerns. Originally, the researchers wanted to collect pre-post surveys directly from the hospital. This would have allowed for the researchers to assess anxiety levels before and after the child's hospitalization to help assess whether the support services helped for those who received them. The researchers were in contact with multiple hospitals to attempt to use their discharge process as a setting to collect data and devised an IRB application for Monmouth University to conduct this research. The IRB would not pass the project because the ethical concerns were too large. Also, with only a year to complete the project, it is possible that once the IRB process reached the hospital it would be too expansive for the time frame. The research departments in corporations as large as hospitals are sometimes too expansive within themselves for a non-affiliated researcher to infiltrate.

It is also more difficult to get approval for any research that involves vulnerable participants. In order to complete research, researchers must complete an NIH training for protection of human participants. This training teaches researchers and students the important role that ethics play in

research. It explains the Belmont Report which was the first document that outlined the ethical principles involved in protection of human subjects. These ethical principles are based on respect for persons, beneficence and justice (National Commission for the Protection of Human Subjects of Biomedical and Behavioral Research, 1978). The training teaches about the ethical dilemmas of conducting research with vulnerable participants. Vulnerable participants are people who are in situations in which they are more likely to find themselves needing assistance. In the case of the present study, both the parent and the child involved in the study are part of vulnerable populations. There would have been even more ethical challenges if the researchers sought to collect data from the children. Just because it is more of a process to conduct research using vulnerable population participants does not mean that the research is less important. If anything, it is more important to help vulnerable populations because of their situations. It is important to hear their voices to better understand their problems.

Although the researchers came across many difficulties in reaching this population, they were invested in the research and persevered to find a way to reach a sample. Once using a hospital as a setting to collect data was no longer a viable option, the researchers moved onto settings that would provide the same population that a hospital would. This led the researchers to Ronald McDonald House. Once the collaboration with Ronald McDonald House of Long Branch was set, a revised survey was devised. The method of collecting data required some changes due to the fact that the data collection was no longer going to be during hospitalization. Ronald McDonald House only has eight rooms for families and there is never a time when all of them are occupied. It is also common for families to stay for long periods of time, which reduces the amount of available participants in any given time frame. For these reasons, the researchers and Ronald McDonald House agreed that it would be easiest to change the method of data collection to be sent to past residents. This would allow for more potential participants but could no longer be done in paper and pen format. The House Manager agreed to send the survey to her database of families from recent years. This was the closest that the researchers were going to get to the original sample that they desired. A new IRB application was created for the project and once it was passed, the survey was converted to an online survey design, and sent out to the participants. The House Manager agreed to send the link every two weeks. Throughout the almost two months that the survey was distributed the one

participant completed the survey at just about the three week mark. The researchers were hoping for, at the very least, fifteen participants. These fifteen participants would have been a small sample, but still provide the data that the researchers needed to explore the research question.

This problem with access to participants is something that needs to be looked into and fixed. The research involving this population is extremely important. The ethical nature of the research is concerning to Institutional Review Boards which makes it harder to find ways to conduct this research.

Future Directions

This is an important topic that needs to be researched. There are many gaps in the literature and the current study did not have the ability to fill these gaps. Future directions should address all of the limitations that were placed on the current study. Since the lack of the participants was the major problem with the study, the first suggestion for future research would be for the researchers to ensure a much larger sample. This can be done by collaborating with more than one Ronald McDonald House. This should not affect the study because even in the same Ronald McDonald House, participants will be offered different support services depending on their case. The survey will allow for many different support services to be explored. The survey should also be conducted in a face to face interview with more room for qualitative data about the support services that the families were offered. This might be most effective if it is done while the participant's child is still being treated at the hospital. It would be preferable if this interview could be done in the last day of the hospital stay or treatment. If it is done any earlier than that it could be flawed because the family could receive support services after the interview that would not be included in the data. Collecting the data at the time of the child's hospitalization would also allow the researchers to use a reliable anxiety measure and assess anxiety at the time instead of asking the parents to reflect on their anxiety in the past. This would be much more effective in exploring how the support services affected the anxiety of the parent.

Future research might also wish to add siblings to the research. If siblings are added the researchers would be provided with a more complete view of all of support services that the family was offered. It is possible that the siblings of the patient are provided with services that the parents are not offered. A new survey would have to be devised in order to look into siblings

because they would need to be asked questions that were relatable to them and less demographic questions. Another variable that could be added is a personality measure. It would be interesting to see if the participant's personality has any effect on the level of anxiety and even the support services that they are offered. Someone who has neurotic tendencies and is not open to new experiences may experience more anxiety than someone who does not have neurotic tendencies and is open to new experiences. People with different personality traits could also respond differently to different support services. When factoring in anxiety level and personality, the support services may have a different effect on parents of hospitalized children. Another variable that would be interesting to explore is marital stress during hospitalization. To assess this variable it would be necessary for both the mother and father to complete the original survey as well as an additional measure targeting marital stress.

Conclusion

The current study aimed to examine the effect of support services on the perceived anxiety level of parents of hospitalized children. It was hypothesized that the support services offered would decrease the perceived anxiety level. It was also hypothesized that a positive relationship between the parents and the doctors would decrease the anxiety level. Mothers were expected to have a higher anxiety level than fathers but also to be more influenced by the support services that were offered. The hypotheses could not be supported due to lack of data to examine since there was only one participant that completed the survey. Although the hypothesis could not be supported, there was results that were examined. The participant suggested that to improve Ronald McDonald House she would add an exercise option; whether it be access to a local gym or an in house cardio option. By using this study as a base, other researchers can delve further into the population of parents of hospitalized children and hopefully find some other ways to improve on the support services that they are offered.

[11] Glenn Gould

[2] Is used to suggest a majestic manner of performance, either in mood or speed.

[3] Composed by Glenn Gould

[4] Atonal music is music that has no specific tonality, is not in a specific key and therefore has no specific 'home' note or chord. The word atonality refers technically to various forms of 20th century music not in a key. *Naxos*.

[5] Rhythm, an essential element in music in one way or another, is the arrangement of notes according to their relative duration and relative accentuation. *Naxos*.

[6] A theme is a complete tune or melody which is of fundamental importance to a piece of music. Naxos.

[7] *It.* Support. An embellishing note or tone preceding an essential melodic note or tone and usually written as a note of smaller size. A grace note.

[8] To shift to another key.

[9] Glenn Gould, Hamburg.

[10] Music between the acts of a play or opera, entr'acte.

[11] "Alecto." *Encyclopedia Mythica* from Encyclopedia Mythica Online. Alecto is one of three Erinyes (furies) who drove their victims mad.

[12] Muskoka, Ontario

[13] A vocalize is a vocal work, whether an exercise or not, that has no words. Naxos.

[14] During Gould's 1957 Tour of Russia, he sent this postcard home to his dog, addressed: Mr. Banquo Gould, 32 Southwood Drive, Toronto.

[15] A quick, improvisational, spirited piece of music.

[16] "Accidental, adj., n., and adv.". OED Online. December 2011. Oxford University Press. Of a note: raised or lowered by one or two semitones, in momentary departure from the key signature; being or marked with a sign indicating this.

[17] Youens, Susan *Schubert's Poets and the Making of Leider*. New York: Cambridge University Press. 1999.

[18] The simultaneous combination of contrasting rhythms in music. Merriam-Webster.

[19] From "Trouble," *All-American Poem*, Matthew Dickman 2009.

[20] *Glenn Gould Reader*, "A Biography of Glenn Gould," p 445.