Modification of a novel polymer with potential use in artificial heart valves: effects on hemocompatibility and mechanical properties

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MODIFICATION OF A NOVEL POLYMER WITH POTENTIAL USE IN ARTIFICIAL HEART VALVES: EFFECTS ON HEMOCOMPATIBILITY AND MECHANICAL PROPERTIES

A thesis submitted in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

in

BIOMEDICAL ENGINEERING

by

Tejas Dilipkumar Choksi

2004
To: Dean Vish Prasad  
College of Engineering  

This thesis, written by Tejas Dilipkumar Choksi, and entitled Modification of a Novel Polymer with Potential Use in Artificial Heart Valves: Effects on Hemocompatibility and Mechanical Properties, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this thesis and recommend that it be approved.

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Florida International University, 2004
DEDICATION

Dedicated to My Loving Family with Love and Adoration
ACKNOWLEDGMENTS

I am truly grateful to everyone who has directly or indirectly supported and helped me complete this M.S thesis. First and foremost, I would like to thank my thesis advisor Dr. Richard Schoephoerster for his advice, mentoring, and providing resources necessary to complete my thesis.

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ABSTRACT OF THE THESIS

MODIFICATION OF A NOVEL POLYMER WITH POTENTIAL USE IN
ARTIFICIAL HEART VALVES: EFFECTS ON HEMOCOMPATIBILITY AND
MECHANICAL PROPERTIES

by

Tejas Dilipkumar Choksi

Florida International University, 2004

Miami, Florida

Professor Richard T. Schoephoerster, Major Professor

Platelet mediated thrombosis has been a major source of mortality and morbidity in cardiovascular device patients as the device surface upon contact with blood induces surface thrombosis. Two specific objectives of this thesis are: 1) Identify the effect of phospholipid modification on Quatromer and 2) Identify the most effective modification technique that will inhibit the surface induced thrombosis and enhance the long-term blood compatibility of Quatromer. Six different phospholipids were grafted in the Quatromer and the role of each of the modified substrates was evaluated in terms of mechanical properties and hemocompatibility. The surface analysis studies showed a significant decrease in contact angle while an increase in the surface energy and the O/C ratio with phospholipid modification, indicating a highly hydrophilic surface with increased carbonyl groups on the surface contributing to lower platelet adhesion and activation. The comparative platelet adhesion tests showed significant decrease in platelet adhesion with phospholipid modification. Hence, phospholipid modification proves to be promising in increasing the long-term blood compatibility of Quatromer.
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CHAPTER I
INTRODUCTION

1.0 Introduction

"The heart, consequently, is the beginning of life; the sun of the microcosm, even as the sun in his turn might well be designated the heart of the world; for it is the heart by whose virtue and pulse the blood is moved, perfected, and made nutrient, and is preserved from corruption and coagulation" – William Harvey (1578-1657)

Heart valve diseases remain a major public health issue. Since their introduction in 1960’s, prosthetic heart valves have significantly improved the quality of life for millions of patients worldwide (Edmunds 2001). According to American Heart Association’s (AHA) 2003 Heart and Stroke Statistical Update, in 2001 an approximate 82,000 valve replacement procedures were performed in the United States. It has been estimated by the American Heart Association’s 2003 Heart and Stroke Statistical Update, that the valvular heart disease is responsible for nearly 19,737 deaths each year in the United States and is a contributing factor in about 42,100 deaths. This indicates a need to introduce improvements in the valve material and design to minimize thrombotic potential and material degradation and to improve morbidity and mortality outcomes (Schoephoerster and Chandran 1991). This study is directed towards the improvements in valve material, which can lead to minimization of thrombotic potential that can eventually help in the development of novel artificial heart valve material.
Heart valve prostheses are basically divided into three groups: 1) mechanical valves, which are fabricated from metal or pyrolytic carbon that directs the flow of blood through tilting or caged discs or with closure of a ball in a cage; 2) bioprosthetic valves, which are tri-leaflet treated aortic valves fabricated from pig (porcine) or pericardium of cows (bovine); and 3) polymer trileaflet valves, which are fabricated from flexible inert synthetic materials. Mechanical valves are structurally designed to last a lifetime, but require potent blood thinners to decrease the tendency to form blood clots (Gray RJ 1996). According to the American Heart Association’s 2003 Heart and Stroke Statistical Update, thromboembolic complications occur with 13-17% of mechanical valves within 5 years of implantation and with 34-44% within 15 years of implantation. Bioprosthetic valves demonstrate advantages in native blood flow characteristics of natural valves, however, have limited durability due to stiffening of calcified leaflets (Schoen 1983; Ozaki 2003). Polymer tri-leaflet valves are constructed from biochemically inert synthetic materials having excellent blood flow characteristics as well as having the prospective to last for a long time (Imamura and Kaye 1977). However, they have not yet been successful due to long-term structural degradation, resulting from oxidative reactions with blood and high tensile and bending stresses originating in the material (Bernacca 1995). These synthetic materials are hydrophobic in nature and they tend to accumulate platelets on the surface, which may later embolize. In particular, the hemodynamic deterioration can result in complete obstruction of the polymer valve, which can develop into a life-threatening condition. Patients with polymer heart valves may have a high risk of thrombus formation on the valve and subsequent systemic embolism (Little S, 2003). These patients therefore need to receive life-long oral
anticoagulation (OAC). The anti-coagulants cause birth defects in the first trimester of fetal development, making the therapy unsuitable for pregnant women. Nevertheless, complications related to thromboembolism and anticoagulant-related hemorrhage is to be considered in deciding polymer heart valve prostheses (Torn 2004). According to the American Heart Association’s 2003 Heart and Stroke Statistical Update, anticoagulant-related hemorrhagic complications of mechanical valves include major hemorrhage in 1-3% of patients per year and minor hemorrhage in 4-8% of patients per year. The mortality rate associated with surgical therapy for valve obstruction is approximately 15%.

The major purpose of this thesis was to investigate the possibilities of developing a new modified polymer, which can be used to fabricate a non-thrombogenic and flexible heart valve leaflet material. The novel triblock polymer is composed of polystyrene-polyisobutylene-polystyrene (Quatromer) copolymers, a proprietary polymer, modified with six different kinds of commercially available phospholipids. It has been found that Quatromer is less likely to degrade in vivo as compared to polyurethane (Pinchuk, Khan et al. 1999). The modification with phospholipid may impart novel properties to Quatromer. Phospholipids present in the outer membrane of human endothelial cell walls have shown to impart great blood compatibility in vivo. The modification of Quatromer may provide a superior biomaterial for trileaflet heart valves. In order to assess the potential of the Quatromer/Phospholipid composite, various methods of modification as well as the mechanical, surface and thrombogenic properties of Quatromer and various types of phospholipid modified Quatromers were analyzed.
2.0 Background

This first part of this chapter provides a general view on the cardiovascular system and anatomy of the human heart. The initial part of the chapter also includes details on the function of the heart, heart valves, the different types of valvular heart diseases, their effects on the heart valve functioning and current treatment options for valvular diseases. The second part of the chapter comprises the history and previous work performed in the field of artificial heart valves. The third part of the chapter includes polymer heart valves, its characterization, different polymers used in heart valves, blood compatibility of polymer heart valves, device hemocompatibility factors, and phospholipid mechanism in polymer modification.

2.1 Cardiovascular System

The cardiovascular system consists of the heart, circulatory system and cells and plasma. The human circulatory system comprises of a network of vessels called arteries, veins, and capillaries, which help transport blood to the various parts of the body and the heart is a pump that pumps blood to the different parts of the body.

2.1.1 Heart

The heart is a strong muscular pump located between the lungs and to the left of the sternum, and weighs between 7 and 15 ounces. The average heart beats 100,000 times, and pumps about 2,000 gallons of blood every day (Martini 2001). The heart wall
is comprised of a double-layered membrane called myocardium, and the layers of the membrane are separated by a layer of fluid, which helps in providing lubricity. The heart contains two upper (left and right atriums) and two lower (left and right ventricles), which are separated by unidirectional valves.

![Anatomy of human heart](http://www.surgery.com/)

Figure 2.1 Anatomy of human heart (Image courtesy of [http://www.surgery.com/](http://www.surgery.com/))

2.1.2 Heart Valves

The human heart has four valves, which control the blood flow in the heart. All four heart valves are unidirectional or one-way valves, which allow the blood to flow only in a single direction. The four types of heart valves are: 1) tricuspid (controls blood flow between the right atrium and right ventricle), 2) pulmonary (controls blood flow from the right ventricle to the pulmonary arteries), 3) mitral (controls blood flow between the left atrium and left ventricle), and 4) aortic (controls blood flow from the left ventricle to the aorta) (Figure 2.1).
Each valve has a set of leaflets also known as flaps. The tricuspid, pulmonary, and aortic valves have three leaflets while the mitral valve has two leaflets. It has been observed that the failures of the heart valves on the left side of the heart are the most common and deadly (Legrice 1997). Hence, the aortic and the mitral valves present on the left side of the heart are subjected to the maximum damage due to higher-pressure differences in the left ventricle of the heart. Thus, in order to develop an artificial heart valve, the structure and function of a natural aortic valve would be studied and mimicked as it represents a case of maximum stress loading in vivo.

2.1.3 Anatomy and function of natural aortic valve

The aortic heart valve primary function is to prevent the blood from flowing back into the left ventricle from the aorta. The aortic valve performs 30-40 million cycles every year, which results in approximately 3 billion cycles in a lifetime (Thubrikar 1990). The above information shows that the fabrication of an artificial heart valves requires careful understanding of the function and material structure of the human aortic heart valve.

The aortic valve contains 90% water and other components such as collagen, elastin, and glycosaminoglycans (GAGs) form an important part of the structure of the valve (Cataloglu 1977; Sacks 1998). The collagen and elastin are the major components responsible for providing the leaflet with its mechanical properties. One of the crucial components for this research study is the glycosaminoglycans, which helps provide the leaflets with required hemocompatibility (Baumann 1997). Studies performed have shown that the hydrophilic glycosaminoglycans have been effective in decreasing protein
deposition and fibrous tissue formation. One of the glycosaminoglycans, hyaluronic acid (HA) has been used extensively in coating medical devices such as polyurethane heart valves, stents, and intraocular lenses. Studies showed that hyaluron modification resulted in good anticoagulant activity, low platelet aggregation, and favored the growth of human endothelial cells (Baumann 1997).

The heart valve leaflets are comprised of three layers of tissue materials (Figure 2.2): 1) fibrosa, 2) spongiosa, and 3) ventricularis (Clark 1974: Sacks 1998). The fibrosa is composed of collagen and is present on the aortic side of the valve. The ventricularis layer is on the ventricle side and is composed of elastin. Spongiosa layer is present between the fibrosa and ventricularis and consists of mainly glycosaminoglycans (GAGs) and some collagen and elastin.

![Figure 2.2 Three layers of aortic leaflet: fibrosa (F), spongiosa (S), and ventricularis (V) (Clarke 1974)](image)

2.1.4 Heart Valve Diseases

In recent years, heart valve diseases have become big threat to human life and one of the primary treatments is surgery. According to the American Heart Association’s 2003 Heart and Stroke Statistical Update, around five million Americans are diagnosed
with valvular heart disease every year and is responsible for nearly 19,737 deaths every year in the United States.

The malfunctioning of natural heart valves can result in several ways. The most frequent causes of valve damage are rheumatic fever, birth or congenital defects, damage from a heart attack, weakening of heart muscles and supporting structures in elderly, and infections such as infective endocarditis (Rahimtoola 2000). The diseases can cause valve damage in two ways: stenosis and regurgitation. Valvular stenosis is characterized by narrowing, stiffening, or blockage of valve thereby preventing free flow of blood. Valvular regurgitation is a condition, which results in improper closing of the valve thereby causing blood to flow in the wrong or back direction. The valves most commonly affected are the aortic and mitral valves as they are subjected to higher-pressure differences (Martini 2001). Thus, this study will focus on mimicking an aortic valve structure and function for the development of a polymer heart valve.

In rheumatic fever disease, fusing of valve leaflets together or calcium deposition on the tissues results in valvular stenosis. Rheumatic fever is responsible for 45% of stenotic valve cases (Mark H. Beers, 17th edition). Congenital or birth defects, endocarditis infection, chronic hypertension causing aortic dilation, heart attack, and autoimmune diseases are some of the causes responsible for valvular regurgitation (Martini 2001). Some birth defects may not be serious enough for a surgery, yet their presence may decrease life expectancy. Birth defects overall cause 20% of the valvular regurgitation cases (Mark H. Beers, 17th edition).

Aortic dilation is another serious condition caused by the enlargement of the sinuses of valsalva, where the leaflets are attached to each other (Butterfield 1993). The
dilation is caused due to conditions such as hypertension or arteriosclerosis. Some extreme cases of aortic dilation may require replacement of the entire valve.

### 2.2 Heart Valve Replacement

The first surgical replacement of a natural heart valve was performed successfully in 1960s. According to the American Heart Association’s *2003 Heart and Stroke Statistical Update*, it is estimated that around 82,000 valve replacements are performed in the United States every year.

#### 2.2.1 Types of Artificial Heart Valves

At present prosthetic heart valves are divided into three groups on the basis of the material from which they are fabricated. The three types of artificial heart valves are mechanical valves (fabricated from pyrolytic carbon or stainless steel alloys), prosthetic tissue valves (fabricated from porcine or bovine pericardium), and synthetic or polymer heart valves (fabricated from inert polymers such as polyurethane). Of the above three classes of valves, only mechanical and tissue valves are commercially available and used in valve replacement at present.

#### 2.2.2 Mechanical Prosthetic Heart Valves

Mechanical heart valves are very durable, highly resistant to structural failure, and can typically last more than 20 years without material degradation (Sapirstein and Smith 2001). They are typically fabricated from biocompatible materials such as pyrolytic carbon or metals alloys such as stainless steel alloys. However, the major drawback of mechanical valves is that the surface tends to attract and activate platelets resulting in
surface thrombosis (Gray 1996). Thus, mechanical heart valves require patients to take anticoagulation therapy indefinitely. However, patients with thrombogenic valvular prostheses, carries a risk not only for valve thrombosis, but also a risk of bleeding associated with anticoagulant therapy (Little 2003). Also, it has been demonstrated that anticoagulation in extra-cardiac surgery, and anticoagulation in pregnancy, involving the risk of embryopathy are some of the major complications associated with anticoagulation therapy (Roudaut 2001). Currently, there are three general designs approved for prosthetic mechanical heart valves: bileaflet, ball and cage, and tilting disc.

One of the earliest designs for mechanical valves was the ball and cage design that uses a small ball entrapped within a metal cage. A typical example of the design is the Starr-Edwards model. The Starr-Edwards model consists of a silicone ball enclosed within a cobalt chromium alloy cage (Matthews 1998). As the blood flows through valve, the flow presses the silicone ball against the aortic side of the cage thereby allowing blood to flow around the ball (DeWall 2000). However, when there is back flow of the blood, the ball is pressed against the ring thereby blocking the flow of the blood in the reverse direction. One of the disadvantages of this heart valve was that the silicone rubber would swell up by absorbing fluids in vivo and therefore would be stuck in the cage. Ball and cage valves exhibit non-central flow characteristics, where the ball blocks the central flow thereby causing the blood to flow around the ball (DeWall, 2000). This results in an increase in stress on the heart, as the heart has to do additional work to compensate for the energy lost due to the change in the direction of the blood. Also, as the ball blocks the flow path of blood, a lot of blood cells are damaged and activated due to the collision
with the ball. This damage of blood cells results in the activation of blood clotting agents, thereby requiring lifelong anticoagulant therapy.

Figure 2.3 A Starr-Edwards Ball and Cage Heart Valve (Image courtesy of http://people.hofstra.edu/faculty/sina_y_rabban/engg81/engg81courseoutline.html)

In the mid 1960s, a new design of mechanical valves was introduced, having a circular tilting disc held in the middle by struts to prevent reverse flow. The Bjork-Shiley model is one of the most famous valves based on this design (Edmunds 2001). The tilting pattern helped in obtaining improved central blood flow characteristics than the ball and cage model. The new model consisted of a tilting-disc fabricated using graphite or pyrolytic carbon and is held together by struts prepared from stainless steel or titanium (DeWall 2000). The disc between the two struts opens when the blood is passing through it while closes when the blood tries to flow in the opposite direction. The tilting design also helped reduce damage to blood cells by reducing their collision with the metal surface due to the central flow characteristics. Yet, one of the major problems with this design is the failure of the struts resulting from the fatigue caused by repeated movement of the struts (DeWall 2000).
In 1979, St. Jude Medical introduced a new kind of mechanical heart valve design known as the bileaflet valve design and today it is the most used design for the manufacture of mechanical heart valves (DeWall 2000). The bileaflet model has two semicircular discs that are held by hinges and have a movement similar to a door. The two discs are fabricated from pyrolytic carbon, having high strength. The greatest advantage of this design was the presence of a larger orifice area compared to the other designs thereby providing the best central flow characteristics (Montorsi 1996). Yet, the major drawback was that the leaflets did not close completely, which caused some leakage of blood in the reverse direction.
2.2.3 Bioprosthetic Heart Valves

Bioprosthetic tissue valves can be basically divided into two categories, namely human tissue valves and animal tissue valves. The tissue valves have many advantages over the mechanical valves as they have better hemodynamics, prevent damage to the blood cells (central flow characteristics) and hence, eliminate the need for a permanent anti-coagulation therapy. However, they suffer from long-term material degradation due to the calcification of the tissue.

Human tissue valves can be classified as: Homografts and Autografts. Homografts also known as Allografts are human valves that are transplanted from a dead donor to a patient (Sapirstein and Smith 2001; Chew et al., 1994). The homografts have various other advantages compared to tissue valves as they provide high resistance to infective endocarditis, native hemodynamic flow characteristics, good durability, and no immunosuppressive treatment, as the organ rejection is minimal (Sapirstein and Smith 2001). However, one of the major drawbacks is the limited availability of donors. The Autografts are human tissue valves that are obtained from the same patient they are to be implanted into. An autograft surgery involves substituting patient’s defective aortic valve with the patient’s own pulmonic valve, and the pulmonic valve with a new artificial valve (Sapirstein and Smith 2001).

Animal tissue valves are also divided into two groups: porcine (pig) valves and bovine (cow) valves (Chew et al., 1994). The tissue components of the heart valve are obtained from either the pericardial tissue of a pig or a cow. The tissue components are kept in glutaraldehyde to increase biocompatibility and to crosslink the collagen fibers thereby improving the strength of the tissue. However, the crosslinking of collagen fibers
has been responsible in causing calcification. The deposition of calcium causes the stiffening of the leaflets, which ultimately leads to the tearing of the leaflets, and may also cause blockage of blood flow (Schoen 1983; Ozaki 2003). Hence, the animal tissue valves are required to be replaced more frequently than mechanical heart valves. The animal tissue valves can be fabricated either with a frame or without a frame (stentless). The stented tissue valves have the valve sewn to a metal or a plastic frame and the frame is then covered with cloth or Dacron. The stentless valves use the patient’s own aortic wall for support (Luciani 2004).

Figure 2.6 A LifeNet Allograft and a Toronto SPV® Tissue Valve (Image courtesy of http://heartvalvebank.info/HVB_valves.php)

2.2.4 Polymeric Tri-leaflet Heart Valves

Polymer heart valves are a new class of heart valves, which have the potential to eliminate the drawbacks present in mechanical and tissue heart valves. The polymeric heart valves are fabricated from biochemically inert synthetic materials, which are designed to have long-term structural and functional stability inside the body (Hyde et al., 1999). The flexible trileaflet structure of the valve enables to provide the combination of superior hemocompatibility (native flow characteristics) and long-term biological and mechanical properties (resistant to oxidation in vivo). They have a potential to eliminate
the anticoagulation therapy required in mechanical valves and provide resistance to material degradation encountered in tissue valves.

Various kinds of polymeric heart valves have been developed and tested both in vitro and in vivo. However, each of them has encountered structural degradation in vivo, which has prevented their commercial success. The next section outlines material degradation faced by different polymers used in fabrication of artificial heart valves in vivo. New significant developments in the field of surface modification of the polymers may help prevent potential problems such as thrombus and calcification, and the early in vitro and in vivo results have been quite promising (Bernacca and Wheatley 1998). One of the most commonly used polymers in polymeric heart valves is polyurethane (Bernacca, Mackay et al. 1995; Bernacca, O'Connor et al. 2002). Polyurethanes have performed well in terms of tensile strength, but have been observed to fail due to oxidation reactions with blood at high bending stresses.

Figure 2.7 Synthetic (Quatromer) Heart Valve (Image courtesy of www.innovia-llc.com)

2.3 Different Polymers in Synthetic Heart Valves

One of the earliest polymers used in polymer heart valves was siliconee rubber, but the material failed under prolong and high fatigue and bending stresses (Chetta and Lloyd 1980; Parfeev, Grushetskii et al. 1982). Another polymer used in the early
developments was Polytetrafluoroethylene, however, the material failed in vivo due to thrombosis and more prominently due to calcium deposits in the commissural region (Nistal et al. 1990).

PGA-PLA (polyglycolic acid/polylactid acid) copolymer was another biomaterial used in fabrication of prosthetic valve leaflets, however this polymer was unsuitable as it was too inmalleable to form flexible leaflets (Stock and Mayer 2001). Studies were also carried out to use PLA-PVA (poly (lactic acid)-g-poly (vinyl alcohol)) polymers in prosthetic heart valve, however the polymer is not yet successful in long term durability studies (Nuttelman et al. 2002). A study carried out by Sodian and his colleagues showed the use of polyhydroxyalkanoates (PHAs), particularly polyhydroxyoctanoate (PHO) in the fabrication of tissue leaflets (Sodian et al. 2000). Polyhydroxyoctanoate (PHO) is biodegradable, biocompatible polyester that proved to be better than the PLA-PGA and PLA-PVA polymers in terms of flexibility, biocompatibility, and mechanical properties; however it also suffered from material degradation over prolonged period in vivo. To overcome the problems faced by PHO, recently Hoerstrup and colleagues developed a new composite material having PGA coated with poly-4-hydroxybutyrate (P4HB), which has a higher degradation time than PHO (Dvorin et al. 2003).

Most recently, Victrex (Lancashire, United Kingdom) has developed PEEK (polyaryletheretherketone) polymer, which is been used by Aortech international to fabricate heart valves. The PEEK valves are currently under clinical studies.

One of the most often used polymers for synthetic heart valves has been polyurethane. Over the past twenty years various forms of polyurethanes have been used in the making of synthetic heart valves (Chetta and Lloyd 1980; Bernacca, Mackay et al.
Polyurethane materials are thermoplastic elastomers having high tensile strength and relatively good biocompatibility, yet they suffer structural degradation due to calcification.

2.4 Mechanism of Blood Coagulation and Device Hemocompatibility

Blood compatibility is one of the most required and desired properties in the development of medical devices. The response of blood in contact with foreign materials can be aggressive, resulting in a highly unfavorable condition such as surface induced thrombosis or blood clots. One of the major risks associated with thrombosis is the detachment of thrombi embolism from the surface of the device and flowing downstream and blocking the smaller vessels thereby blocking the vessels, which may result in severe complications such as stroke or even death. According to AHA’s 2003 Heart and Stroke Statistical Update, thrombosis and thromboembolism complications results in approximately 4-20% of prosthetic heart valve patients. Hence, it becomes vital to evaluate and diagnose the thrombotic potential of a biomaterial before it is introduced in the device fabrication phase.

2.4.1 Blood Coagulation

Blood is composed of various types of cells, platelets, and proteins known as blood coagulation factors, which are numbered from I-XIII (Guyton and Hall 1996). When the body suffers an injury, it initiates a process called homeostasis to stop bleeding by the mechanism of blood coagulation. However, when this blood comes in contact with external materials, the coagulation factors are activated and an enzyme called Thrombin is secreted. Thrombin is then responsible in converting the protein fibrinogen into an
insoluble product known as fibrin. This fibrin is the main component, which helps in the aggregation of blood cells and platelets (Colman 1994; Grunkemeier 2000). The aggregates of activated platelets tend to join each other on the surface thereby forming fibrin clots. Hence, when a medical device comes in contact with blood, one of the inactive proteins called factor XII is adsorbed on the foreign surface. Upon deposition on the foreign surface, the factor XII becomes active to factor XIIa and it begins the cycle of coagulation (Blockmans 1995; Grunkemeier 2000). The activated factor XIIa is needed for the cross linking of fibrin molecules. As more and more fibrinogen is converted to fibrin, a network of fibrin is created in the presence of factor XIIa, which entraps platelets on the surface of the material. The platelets on attaching to the surface become activated, thereby releasing chemicals, which attract other platelets in the vicinity, inducing the formation of platelet aggregates. The aggregated platelets on the surface produce more thrombin inducing further fibrinogen to fibrin conversion, thereby aggravating the coagulation process (Colman 1994; Blockmans 1995; Grunkemeier 2000).
2.4.2 Device Hemocompatibility

Every device has the potential to form thrombosis on the surface by the presence of various clinical and design factors. The various factors responsible for the hemocompatibility of medical devices are blood flow dynamics, device geometry, surface roughness, medical device material, duration of blood contact, and surface area (Vroman 1988; Hirsh 1983; Ratner 1996).

2.4.2.1 Blood Flow Dynamics

The blood flow controls such as blood shear rates, and turbulence are important factors affecting the amount as well as the composition of blood proteins transported to the device surface. The level of shear stress plays an important role in the adhesion as well as the activation of the platelets. At low flow rates, a red thrombus is formed, which contains a large amount of fibrin binding platelets and red blood cells. The formation of red thrombus results at low flow rates as there is enough time for the proteins to bind to fibrinogen in order to completely bind the platelets (Harker 1974). When the medical device is kept in a high flow, a white thrombus is formed and it contains a large amount of platelets bonded by smaller amounts of fibrin (Harker 1974). The formation of white...
thrombus results at higher flow rates as there is limited time for the coagulation cycle to form fibrin while the platelet cycle takes place, as it only requires an adhesion site for the activation of the platelets (Harker 1974; Slack 1993). In cases of flow disturbances or turbulence in blood flow (arterial stenosis, and vessel bifurcations), slow shear recirculation regions would form, which would result in the formation of a large amount of fibrin (Harker 1974). Thus, turbulence would result in an increase in the activation and transportation of platelets and coagulation factors.

2.4.2.2 Device Geometry

The geometry of the device can be a major contributing factor to determine the blood flow characteristics. The surface (sharp curves and bifurcations) of medical devices may result in turbulence in the blood flow thereby causing aggravated platelet adhesion and activation at the surface (Vroman 1988; Ratner 1996).

2.4.2.3 Surface Roughness

The degree of surface roughness plays a vital role in contributing towards the device hemocompatibility (Brash 1987; Horbett 1993; Andrade 1996). It is observed that rough surfaces tend to have an attacking nature and hence, they tend to accumulate more platelets on the surface. The irregular rough surface results in the formation of micro-turbulence areas, thereby making it more susceptible to attract and activate platelets on the surface. Platelet studies performed on polished surfaces having smoother surfaces tend to show lower thrombogenic potential than unpolished ones (Andrade 1996).
2.4.2.4 Medical Device Material

The surface chemistry of the device material is very important in providing the device with its blood compatibility. Various surface properties such as degree of hydrophilicity, surface energy, surface charges (anionic or cationic), and crystallinity contribute to the hemocompatibility of a medical device (Vroman 1988; Hirsh 1983; Ratner 1996). For the device material to be highly hemocompatible, ideally it should be highly hydrophilic, non-ionic, inhomogeneous, non-crystalline, and have high surface energy.

2.4.2.5 Duration of Blood Contact

The time for each a device is in contact with blood can help determine the reaction of the blood with the device material (Hirsh 1983). Time has a direct relation with platelet deposition i.e. the more the material contact time with blood, the greater the amount of platelets deposited on the surface. Heart valves, which are typically implanted for years, may result in systemic effects such as infection, anemia or calcification over time due to the presence of proteins layers on the device surface (Vroman 1988).

2.4.2.6 Surface Area

Surface area is also directly proportional to amount of platelets deposited on the surface. The greater the material contact surface exposed to platelets, the more platelets attached to the surface. Medical devices having large surfaces can result in conditions such as anemia as a lot of red blood cells are attached on the surface thereby reducing the amount of red cells flowing in the blood (Vroman 1988).
2.5 Hemocompatibility of Polymers in Vivo

Polyurethane materials have been the typical choice for biomedical implants due to their high tensile strength, and good hydrodynamic flow characteristics (Bernacca 2002). The polyurethane materials have also been shown to survive 400 million cycles in fatigue testing (Jansen and Reul 1992), yet they have not been proved to be hemocompatible under in vivo conditions, which has limited its use in medical applications. Various mechanical testing studies performed on polyurethanes (PUs) show that the mechanical properties (fatigue resistance, toughness, flexibility, and durability) of polyurethanes meet requirements for clinical applications, yet when the polyurethanes come in contact with blood, activation of platelets on the polyurethane surface occurs, which decreases its long-term hemocompatibility (Morimoto et al. 2002). Hemocompatibility studies carried on polyurethanes showed that the hemocompatibility of the polymer correlated closely with hard segment content and chemical composition of the polymer (Groth and Klosz 1994). Platelet deposition studies showed that the adhesion and activation of plasma proteins, lymphocytes, and platelets increased with increasing hard segment content of the polyurethane (Chen and Wei 1998; Mitzner and Groth 1996).

When the blood compatibilities of soft segments of polyurethanes (poly (butadiene), poly (isoprene)) and hard segments of polyurethanes (1,4-butanediol) were evaluated using platelet-rich plasma, it was found that the soft segments of polyurethanes (poly (isoprene)) exhibited lower platelet adhesion (Li et al. 1996). The various platelet deposition studies performed on polyurethanes have showed that they have a high potential to adhere platelets on their surface.
For the fabrication of the novel trileaflet heart valve, a proprietary polymer, called Quatromer (Innovia LLC, Miami, FL), which is a polystyrene-polyisobutylene-polystyrene triblock copolymer is selected. Basically, the polymer is composed of a gummy material i.e. polyisobutylene restricted between two hard blocks i.e. polystyrene. It has been found that the Quatromer is highly resistant to oxidation in vivo due to the absence of ester and ether bonds as well as the backbone of the polymer has sites that can support double bond formation (Pinchuk et al., 1988). However, platelet adhesion studies carried out on polystyrene surface have shown that the polystyrene surfaces have high reactivity for platelet adhesion (Carretero et al. 1994). The platelet studies performed on polystyrene have shown that the hydrophobic nature of polystyrene is one of the vital factors in attracting platelets on the surface. Recently, platelet deposition studies performed on Quatromer and polyurethane have shown that they have equal platelet deposition on their surfaces. This shows that the Quatromer has a high potential to attract platelets on its surface as studies performed on polyurethane have shown high number of platelets adhering to the surface.

Thus, modification of the Quatromer is required to enhance its resistance to thrombosis. One of the methods to reduce the thrombotic potential of the Quatromer is to modify it with phospholipid. The phospholipids tend to bind water loosely around them thereby increase the amount of water molecules on the polymer surface, and thus, decreasing the platelet adsorption on the surface.
2.6 Preliminary work performed in modification of Quatromer

One of the most vital objectives of this thesis is to increase the hydrophilicity of the Quatromer. Quatromer is basically composed of polystyrene and polyisobutylene copolymers and both the materials have been shown to be significantly hydrophobic in nature. Experiments carried out by Carretero have shown that hydrophobic polystyrene surfaces tend to have high potential for platelet adhesion. Also, the surface analysis of Quatromer surface performed using sessile water drop method has shown that the Quatromer surface has a high contact angle indicating high hydrophobicity of the Quatromer material. In order to increase Quatromer’s surface hydrophilicity, a range of surfactants was selected. The contact angle and surface energy of each of the surfactant modified Quatromers were determined to assess the potential of the surfactant in lowering the contact angle of Quatromer. Various surfactants namely sotradecol (sodium tetra decyl sulfate), n-butyl methacrylate, Alginic acid, Acrylic acid, Phytic acid, Poly(L-lactide), Hyaluronic acid, Didodecyldimethylammonium bromide, Heparin, Phosphatidylcholine, Phosphatidylethanolamine, 1,2-Dimyrystoyl-rac-glycero-3-phosphocholine, and 1,2-Didecanoyl-rac-glycero-3-phosphocholine were used initially to modify the Quatromer and the surface analysis was carried out for each of them. The contact angle measurements showed that the phospholipids tend to show the smallest contact angles indicating their potential in increasing the hydrophilicity of the Quatromer. The contact angle measurements helped us to narrow the range of surfactants used to modify the Quatromer to just phospholipids. After deciding that phospholipids would be the best choice for modifying Quatromer, two new phospholipids 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine, and 1,2-Diarachidonoyl-sn-Glycero-3-Phosphocholine were
added to evaluate the effect of degree of phospholipid unsaturation and phospholipid chain length on hydrophilicity and platelet deposition on Quatromer surface. Thus, in total six different saturated and unsaturated phospholipids were selected to modify the Quatromer and to evaluate the role of each in enhancing the long-term hemocompatibility of the Quatromer.

2.7 Previous work performed in phospholipid modification of polymers

Phospholipids are found predominantly in the outer membrane layer of the cell. They are zwitterionic molecules, which carry both positive and negative charges with a net charge of zero. They zwitterionic nature helps increase the free water on the surface thereby reducing surface thrombosis. This unique property of phospholipids has helped them achieve recognition as the typical material of choice for modifying polymers to increase their blood compatibility (Hayward and Chapman 1984). Since the last 20 years, many studies have been carried out on modifying different polymers using different types of phospholipids.

To achieve novel hemocompatible polymer surfaces, polyurethanes were modified with 2-methacryloyloxyethyl phosphorylcholine (MPC) to form a polymer network. Platelet adhesion studies showed that unmodified polyurethanes had a high level of platelet attachment before and after stress loading while MPC modified polyurethanes prevented platelet adhesion even after stress loading (Ishihara et al. 2000; Morimoto et al. 2002). On analyzing the polymer composite, it was found that the MPC unit was concentrated at the surface of the polyurethane membrane, which organized itself into a self-assembled membrane and thus contacted with the plasma proteins (Ishihara et al.
Studies on poly (ether urethane) (PEU) modification using dioleoyl phosphatidylcholine (DOPC) demonstrated that the blood compatibility of PEU was improved by embedding phospholipid groups on the surface. The improved hemocompatibility was indicated by a decrease in platelet activation and adsorption on the PEU surface (van der Heiden AP 1998). The data from the study showed that the contact angle of the phospholipid modified polymer decreased thereby increasing the hydrophilicity of the surface. One of the main points that the study highlighted was the fact that the presence of mere phospholipid groups was not sufficient in reducing the platelet adhesion, rather the formation of "biomembrane-like" DOPC bilayer was responsible for the reduction in platelet adhesion (van der Heiden AP 1998).

Platelet studies performed on methacryloyloxyalkyl phosphorylcholine (MAPC) polymer showed that the addition of dimyrstoyl phosphatidylcholine (DMPC) further reduced the amount of fibrinogen absorbed on the MAPC surface (Iwasaki Y et al. 1999). The modification of L-lactide polymer using L-alpha-glycelophosphorylcholine (LGPC) resulted in the formation of a biodegradable polymer having good hemocompatibility and antiadhesive properties (Iwasaki Y et al. 2003). The studies mentioned above shows that phospholipids have a great potential in rendering polymeric materials with high hemocompatibility. To achieve the biomembrane properties, the biomedical Quatromer will be modified using different phospholipids to promote the creation of functional groups, such as phosphate, and carbonyl groups. The modifications are expected to enhance Quatromer’s long-term implant hemocompatibility as well as impart other material properties such as reduced surface friction and tackiness.
2.8 Proposed Phosphatidylcholine (PC) Mechanism

Phosphatidylcholine (PC) belongs to the group of phospholipids and it is basically a fat derivative where the head group has one of the fatty acids replaced by a phosphate group. Phosphatidylcholine molecule is amphiphilic i.e. it has a hydrophobic tail (fatty acid end not attracted to water) and a hydrophilic head group (phosphate group attracted to water). Phospholipids have a unique property to self-assemble to form bilayers (Figure 2.10) in aqueous environment where the hydrophilic phosphate head group orients towards the outside while the hydrophobic tail points inwards (Campbell 1994). These bilayers having hydrophilic head groups are the predominant factors responsible for the hemocompatibility of biomaterials (van der Heiden et al 1998).

Figure 2.9 Phosphatidylcholine self-assembling bilayers and head group water-binding mechanism (Image courtesy of www.biocompatibles.co.uk/)

One of the proposed mechanisms, which help Phosphatidylcholine in reducing platelet adhesion on polymer substrate, is presented in this paragraph. When phosphatidylcholine comes in contact with water, it possibly assumes a hydration state where the hydrophilic head groups bind free water around it through vanderwaal forces (Ishihara 1998) (Figure 2.10). It is believed that when a protein comes in contact with the
surface of the polymer, the protein tends to loose its solvation water thereby attaching the hydrophobic part of the protein to the polymer surface. However, when the polymer is modified with phosphatidylcholine, the phosphate head group is supposed to tightly bind free water molecules around it. The presence of these free water molecules around the head groups is believed to cause the state of water molecules on the polymer surface to be the same as the one present around the proteins and this possibly prevents the proteins from releasing their solvation water molecule (Yianni 1992). Therefore, the failure of the protein to release their solvation water molecule is one the possible vital factors, which prevents the hydrophobic interactions between the protein and the polymer surface (Figure 2.11). Hence, phosphatidylcholine modified polymers possibly induces presence of free water molecules on the polymer surface thereby restricting the protein hydrophobic interactions and thus improving the hemocompatibility of the modified polymer (Dudley et al. 1993).

Figure 2.10 Proposed PC mechanism in preventing protein biomaterial interaction
(Image courtesy of www.biocompatibles.co.uk/)
2.9 Types of Phospholipids used in this research study

a. Phosphatidylcholine (Lecithin)

Figure 2.11 Phosphatidylcholine Structure

Phosphatidylcholine (PC) also known as 1,2-Diacyl-sn-Glycero-3-Phosphocholine is one of the most known member of the fat-soluble substances known as phospholipids. PC is an unsaturated phospholipid with a numerical formula of 18:1\textsuperscript{19} which denotes a hydrocarbon chain with 18 carbon atoms and one cis double bond between the ninth and tenth carbon atom. PC is the most abundant phospholipid found in animals and plants. Phosphatidylcholine is the major component in the formation of the outer layer of the plasma membrane of the cells. It is a zwitterionic (neutral) molecule i.e. it has positive and negative charged groups with a net charge of zero. Lecithin is believed to help in fighting arteriosclerosis, digestion of fats, immune disorders, and liver disorders.

b. Phosphatidylethanolamine (Cephalin)

Figure 2.12 Phosphatidylethanolamine Structure
Phosphatidylethanolamine (PE) is nitrogenous unsaturated fatty acids and they are the second most abundant phospholipids found in living organisms. PE is an unsaturated phospholipid denoted numerically as \(18:2^\Delta_{9,12}\) having 18 carbon atoms and two cis double bonds at the ninth and twelfth carbon atoms. PE also forms one of the major building blocks of the cell membrane, especially microbial membranes. Phosphatidylcholine is also a zwitterionic or neutral molecule having polar and non-polar groups. They play an important role in the assembling of membrane proteins and also help in the growth of endothelial cells.

c. 1,2-Dioleoyl-sn-Glycero-3-Phosphocholine (DOPC)

![Figure 2.13 DOPC Structure](image)

This phospholipid has an 18-carbon unsaturated fatty acid having one cis double bond (oleic acid) at the ninth carbon atom in each hydrocarbon chain and is denoted as \(18:1^\Delta_{9,9}\). The double bond is responsible in producing a twist or a kink in the hydrocarbon chain.

d. 1,2-Diarachidonoyl-sn-Glycero-3-Phosphocholine (DAPC)

![Figure 2.14 DAPC Structure](image)
This is a 20-carbon unsaturated fatty acid having four cis double bonds (arachidonic acid) and is designated as 20:4\textsuperscript{5,12,15,18}. It is one of the phospholipids, which forms a major constituent in prostaglandins. This phospholipid is highly susceptible to oxidation due to the presence of a high degree of unsaturation in the hydrocarbon chain. The double bonds are susceptible to cleavage in vivo forming free radicals or peroxides which are highly unstable in nature.

e. 1,2-Dimyristoyl-sn-Glycero-3-Phosphocholine (DMPC)

![Figure 2.15 DMPC Structure](image)

This is one of the most basic and saturated phospholipid having 14 carbon atoms and no double bonds in the hydrocarbon tail. The phospholipid is denoted as 14:0 numerically, and has a highly ordered and stable structure. The absence of the double bond helps this phospholipid in forming a more compact bilayer arrangement. This phospholipid has been widely used in the modification of different polymers.

f. 1,2-Didodecanoyl-sn-Glycero-3-Phosphocholine (DDPC)

![Figure 2.16 DDPC Structure](image)
1,2-didodecanoyl-sn-Glycero-3-Phosphocholine (DDPC) also known, as 1,2-Dilauroyl-sn-Glycero-3-Phosphocholine (DLPC) is a 12-carbon atom saturated fatty acid without any double bonds in the structure and is denoted as 12:0. This phospholipid has a highly ordered structure and is highly hydrophilic in nature.

2.10 Statement and Significance of Problem

Blood compatibility has been the major concern in the development of artificial heart valves since foreign surfaces induce thrombosis upon contact with blood. Heart valve prostheses have been used successfully for half a century in improvement in the longevity of patients with valvular diseases (Edmunds 2001). According to the American Heart Association AHA’s 2003 Heart and Stroke Statistical Update, heart valve mortalities in 2001 were around 19,737, which are still pretty high. Thrombosis and thromboembolism have been major sources of mortalities in cardiovascular devices and hence, efforts are required to introduce improvements in artificial valve material and design to minimize thrombotic potential and material degradation and to improve the morbidity and mortality outcomes (Schoephoerster and Chandran 1991). It is therefore necessary to diagnose and evaluate the blood compatibility of foreign materials before they are considered in the development of artificial organs. Modified synthetic polymer heart valves have shown potential to reduce the thrombogenicity and structural degradation associated with the prosthetic heart valves. Quatromer polymer trileaflet valve offers natural valve hemodynamics with the potential for sufficient durability for long-term use. However, hemocompatibility of synthetic polymers has been one of the limiting factors in the development of polymeric medical devices and artificial organs
due the interaction of blood upon contact with hydrophobic polymer surface, resulting in surface thrombosis.

Patients with polymer heart valves may have a high risk of thrombus formation on the valve and subsequent systemic embolism. These patients therefore need to receive life-long oral anticoagulation (OAC). Nevertheless, complications related to thromboembolism and anticoagulant-related hemorrhage is to be considered in deciding polymer heart valve prostheses. Modern oral anticoagulant therapy is found to be far from ideal. Every patient, with thrombogenic valvular prostheses, carries a risk not just for valve thrombosis, but also a risk of bleeding which follows anticoagulant therapy (Little S, 2003). Also, it has been found that anticoagulation in extra-cardiac surgery and anticoagulation in pregnancy are some of the major complications associated with anticoagulation therapy (Roudaut R, 2001). These complications associated with valvular prostheses and anticoagulation therapy requires efforts to develop a new modified material and technique to reduce or possibly eliminate the complications associated with artificial heart valves.

As a result of the numerous complications associated with valvular prostheses, decreased incidence of thromboembolic complications can be achieved by improved thromboresistance of the new valve material rather than anticoagulant therapy. A polystyrene-polyisobutylene-polystyrene (Quatromer) triblock copolymer, a novel proprietary polymer is being used in the development of the synthetic heart valve. It has been found that Quatromer is found to be less likely to degrade in oxidative and acidic environments than Polyurethane (Pinchuk, Khan et al. 1999), which are the typical conditions likely to be encountered in vivo. However, it is unclear whether Quatromer
valves will suffer from some of the complications associated with the standard valves such as surface thrombosis.

In order to evaluate the thrombotic potential of Quatromer and the feasibility of a modified novel Quatromer as heart valve material, the surface characterization, mechanical properties, and platelet adhesion studies for Quatromer would be compared with six different phospholipid modified Quatromers. Extensive research work has been carried out on phospholipid modification of polymers, yet there are no comparative studies performed, which highlight the effects of different saturated and unsaturated phospholipids having varying hydrocarbon chain length on the same polymer. This research study involves saturated phospholipids (1,2-Dimyristoyl-rac-glycero-3-phosphocholine (DMPC), and 1,2-Didodecanoyl-rac-glycero-3-phosphocholine (DDPC)), unsaturated phospholipids (Phosphatidylcholine (PC), 1,2-Dilinoleoyl-sn-Glycero-3-Phosphocholine (DOPC), and 1,2-Diarachidonoyl-sn-Glycero-3-Phosphocholine (DAPC)), and nitrogenous phospholipid (Phosphatidylethanolamine (EPC)). Basically, the Quatromer is modified with the saturated, unsaturated, and nitrogenated phospholipids and the effect of phospholipid acyl chain unsaturation, chain length, and presence of amine groups on hemocompatibility, material strength, and oxidation resistance of the Quatromer is determined. The study compares the effect of each of the different phospholipids on a single polymer i.e. Quatromer and this distinguishes this research from other phospholipid modification studies. Moreover, Quatromer is a newly invented elastomer, and this would be a first of a kind modification study for the Quatromer thereby evaluating and comparing the hemocompatibility of the Quatromer as well as different surfactant modified Quatromers. Most of the research
studies carried out on the modification of polymers performed either the 1) surface characterization tests or 2) platelet adhesion tests or 3) mechanical tests for the newly modified polymers, however, this study compares all the three above mentioned aspects of polymer evaluation. Research studies have been performed using some of the above mentioned phospholipids such as 1,2-Dilinoleoyl-sn-Glycero-3-Phosphocholine (van der Heiden AP 1998), however the studies were performed on other polymers such as poly (ether urethane) (PEU) and the study also did not compare different phospholipids on the same polymer as well as the did not involve mechanical testing of the modified substrates. Hence, the above mentioned aspects would help in distinguishing this study from others as well as in evaluating the thrombotic potential of Quatromer and modified Quatromer in the fabrication of a novel trileaflet heart valve.
CHAPTER III

METHODOLOGY

3.0 Methodology

The materials and methodology used in this study are divided into three groups, each of which represents the different phase of testing. The first phase of testing is the mechanical testing of the specimens. The second phase is comprised of the surface characterization of polymers. The last phase is the comparative thrombogenic test or platelet adhesion test of the polymers.

3.1 Sulfonation of Quatromer

A proprietary process (Innovia LLC) of sulfonating the Quatromer (lot#09200) was employed to determine its potential in enhancing the hemocompatibility of the Quatromer surface. The sulfonation technique was used to create negatively charged $\text{SO}_3\text{H}$ groups on the surface. The sulfonation process for Quatromer is not disclosed due to the proprietary nature. However, in order to confirm the generation of sulfonate groups on the Quatromer surface, methylene blue staining test was performed. On staining the surface with methylene blue dye, if the surface turns blue in color then it shows the presence of $\text{SO}_3\text{H}$ (sulfonate groups) on the Quatromer surface while the lack of staining would prove the absence of sulfonate groups on the surface (Pizzolato 1975). Basically, methylene blue is a cationic sulfonate sensitive dye and when used to stain a sulfonated polymer, the anionic sulfonated polymer forms ion pairs with the cationic methylene blue dye. Thus, the sulfonated polymer forms a blue colored complex with the methylene dye.
and this helps in staining the surface of a sulfonated polymer blue while an unsulfonated polymer does not form blue colored complex with methylene blue dye (Pizzolato 1975).

### 3.2 Phospholipid/Quatromer modification

The phospholipid modified Quatromer was prepared by surface dip coating process as well as using a simple phase mixing process.

The surface modification of Quatromer is carried out using a simple dipping process. Dip coating of polymers is a simple process, which basically binds the surfactant to the desired substrate. The method is quick, easy, and is relatively inexpensive. Rectangular samples of Quatromer were prepared using a plate assembly (Appendix A) and the samples were then dipped in a 20% (w/w) phospholipid/Quatromer composite. A 20% (w/w) solution of phospholipid/Quatromer composite was prepared by mixing 20% of the desired phospholipid by weight with 20%Quatromer solution in Toluene i.e. to prepare a 5 gram solution of 20%(w/w) phospholipid/Quatromer composite, 200 mg of desired phospholipid was added to 5 grams of 20%Quatromer in Toluene solution (containing 1 gram of Quatromer and 4 grams of Toluene) (Appendix A). The mixture is then stirred on a magnetic stirrer for 15 minutes for the phospholipid to mix homogenously with the polymer. After the mixture is ready, the dog-bone Quatromer samples were dipped in the 20% phosholipid/Quatromer solution for a period of 3-10 seconds. The dipped specimens were then dried in an oven at 50°C for 1 hour. The pull out velocity for the process was in a range of 4.5-15 mm/sec and the thickness of the samples was ranging from 15-30 μm. The results for the dipping process varied for each
batch as the process was performed manually and there was no automation involved to control the pull out velocity as well as the dipping time.

![Dipping coating phospholipid modification process for Quatromer](http://www.solgel.com/articles/Nov00/mennig.htm)

Figure 3.1 Dipping coating phospholipid modification process for Quatromer (Image Courtesy of http://www.solgel.com/articles/Nov00/mennig.htm)

The second method of modification of Quatromer with phospholipid involved a process of mixing the phospholipid with Quatromer by weight to prepare a phospholipid/Quatromer composite. A 3% phospholipid/Quatromer composite was prepared by mixing 3% of phospholipid by weight with a 20% Quatromer solution in Toluene (Appendix A) i.e. to prepare a 3% phospholipid/Quatromer composite, 30 mg of desired phospholipid was added to 5 grams of 20%Quatromer in Toluene solution (containing 1 gram of Quatromer and 4 grams of Toluene). The 3% mixture was selected as studies have shown that 3% phospholipid concentration in polymer material is an optimum amount required to distribute the phospholipid homogeneously throughout the polymer, and imparting hemocompatibility properties (Lin-Yue et al.1997; Muhannad Jumaa et al. 2000). The mixture prepared is poured into a rectangular cavity plate.
assembly (Figure 3.2) to form rectangular specimens (Appendix A). The plate assembly was then allowed to dry in an oven at 50°C for 3 hours.

For all the mechanical, surface characterization, and platelet adhesion tests performed, only the 3% phospholipid/Quatromer composite samples were used in the tests while none of the samples fabricated using the 20% phospholipid/Quatromer dipping process were used to perform any of the above tests. Prior to performing all the mechanical tests, surface characterization tests, and platelet adhesion tests, it was determined that the phase mixing technique has more advantages than the dipping process, and hence, all the various tests were performed with the samples fabricated using the 3% phospholipid/Quatromer phase mixing technique. The details for selecting the phase mixing process over the dipping process are presented later in the discussion section.

3.3 Mechanical Testing

The mechanical testing phase included three different types of testing i.e. tensile testing, peeling strength test, and shear strength test. The tensile tests were performed to determine the stress strain data for Quatromer and the six modified Quatromers as well as to determine the tensile strength of different concentrations of phospholipid/Quatromer composites. The peeling and shear strength tests were performed to determine the tackiness or stickiness as well as the surface (rough or smooth) of Quatromer and the phospholipid modified Quatromer.
3.3.1 Dog-bone specimen preparation

Dog bone shaped specimens were prepared for Quatromer as well as for six different phospholipid modified polymers to perform the mechanical tests (Appendix A). A 20% Quatromer solution was used to prepare the specimens. The Quatromer solution was prepared by mixing 20% of Quatromer pellets in 80% of Toluene solvent by weight. The mixture was then stirred on a magnetic plate for 2-3 hours. Before making the solution, the Quatromer pellets were dried overnight at 70°C in an oven. A stainless steel three-plate assembly was used to cast the polymer solution into rectangular specimens. The plate assembly basically consisted of three components i.e. a stainless steel bottom plate for support, brass gaskets of 0.3 mm thickness, having a rectangular cavity in the center, and stainless steel blocks, also having rectangular cavities in the center which are of the same dimensions as those in the brass gaskets. The brass gaskets were placed on the top of the bottom plate and then the stainless steel blocks were kept on top of the gaskets in such a way that the cavities in each of them were coincident with each other. The three components were held in place by tightening them with six #4-40 screws. Before pouring the solution in the cavities, the cavities were cleaned with alcohol to remove any impurities present on the surface. Experiments were carried out to determine the relation between the quantity of solution poured in the cavity and the specimen thickness, showed that 5 mL of solution gave a desired thickness of 0.3 mm. Hence, 5 mL of polymer solution was poured in each of the cavities and the plate assembly was then placed in an oven at 70°C for four hours. After the samples were dried, they were removed carefully from the plate. A dog-bone shaped steel cutting dye and a hammer was used to slice the rectangular specimens into dog-bone specimens according to a modified
version of ASTM standard D 638-89. The cutting process was then followed by a specimen examination process where the thickness of each of the specimens was measured. The specimens were measured at the two ends as well as in the center, and an average of all the three measurements was recorded. Specimens with average thickness of 0.3mm were considered while the ones with a thickness variation of more than 10% were discarded.

Figure 3.2 Three-plate assembly used to mold rectangular specimens for mechanical tests and dog bone specimen dimensions

3.3.2 Tensile testing

Two sets of tensile tests were performed. The first tensile test was carried out to compare the stress strain data between the Quatromer, and the six different phospholipid modified Quatromers. The second test was performed to evaluate the change in tensile strength by varying the percent weight of phospholipid in Quatromer. The tensile tests of Quatromer and the modified ones were carried out using an Electroforce™ (ELF) 3200 materials tester (Enduratec Systems Corp., Minnetonka, MN). The tensile tests of all the materials were performed according to ASTM standards D 882 – 88 (thin plastic sheets), and D 3039 – 89 (composites). For each sample, at least five specimens were tested. The
dog-bone specimens with thickness of 0.3 ± 0.01 mm were clamped to grips of the machine and were pulled at a speed of 5 mm/min. The results from the tests helped in providing stress strain data i.e. ultimate tensile stress (UTS), ultimate strain (US), and Young’s Modulus (E).

Figure 3.3 EnduraTec ElectroforceTM (ELF) 3200 materials tester

3.3.3 Peeling Test

The peeling test is a secondary and an additional test performed to determine the effect of phospholipid modification on the stickiness as well as the interfacial adhesion between two Quatromer as well as two modified Quatromer surfaces. The peeling tests were carried out according to ASTM standard D 903–98 (Peeling or Strip test). A T-peel test was performed where the angle of peeling was 90 degrees. For the peeling test, two 30 mm long and 0.3 mm thick rectangular strips were prepared using the plate assembly and the polymer strips were then casted on each other over a contact length of 25mm. The top layer of the polymer was laid on the bottom layer in such a way that no air bubbles were trapped between them. One free end of each of the strips was held by the two action grips of the Enduratec machine and was pulled at a rate of 5mm/min force.
The test helped in determining the peel force required to separate the two strips of Quatromer as well as modified Quatromer polymer.

Figure 3.4 Specimen arrangement on Enduratec machine for the Peeling test

3.3.4 Shear Strength Test

The shear strength test is also an additional test performed to evaluate the stickiness and interfacial adhesion forces for plain Quatromer and phospholipid modified Quatromer. The shear strength test was carried out according to ASTM standard D 5321–92 (Interface Shear test). A 180-degree shear bond strength test was performed. Two rectangular polymer strips 0.3 mm thickness and 30 mm length were prepared using the plate assembly and the strips were then cast on each other over a contact length of 10 mm. The two free ends of the specimens were then pulled at rate of 5mm/min by the action grids. The tests helped in determining the interfacial forces between the two polymer surfaces.

Figure 3.5 Specimen arrangement on the Enduratec machine for shear strength test
3.4 Surface Analysis

The surface characterization or surface analysis study consists of the measurements of contact angle, surface energy, the O/C ratio, and the surface texture. The contact angle and surface energy measurements help evaluate the hydrophobicity the Quatromer surface and different phospholipid modified polymers. The O/C ratio study of the biomaterials help analyze the oxygen content as well as the number of carbonyl groups present on the surface which correlate with the number of negatively charged sites and endothelial cell growth on the surface (Ertel 1990).

3.4.1 Contact Angle Measurement

Contact angle measurements of liquids on polymer surfaces are one of the most widely used methods to determine the hydrophilicity and the adhesion properties of solids. The contact angle measurement test was performed according to the ASTM standard D5946-96 (Polymer films). The contact angle or wetting angle is the one included between the tangent plane to the surface of the liquid and the tangent plane to the surface of the solid, at a point of contact. For these experiments, the liquid used to measure the contact angle on the polymer surface was distilled water. The contact angle experiments were performed using distill water rather than other liquids such as blood and alcohols, as using blood would have involved complications related to coagulation (requiring anticoagulants) while alcohols may induce chemical reaction with the Quatromer surface. Also, distill water is one of the most standard and most referenced liquid used for contact angle measurements. The contact angle was measured using a laboratory microscope fitted with an assembly. The assembly basically constituted of a
stage mounted with a triangular glass prism and a glass slide held by two pins. The specimen was kept on the glass slide and a drop of distilled water was placed on the specimen. A TV monitor was connected to the microscope, which helped to show the picture of the drop on the specimen surface. The light from the source passed through the drop of liquid and then struck the prism, which in turn refracted the path of the light to the lenses. The microscope has a 360° scale, which helped to measure the contact angle of the specimen.

Figure 3.6 Contact angle for a solid-liquid interface (Image courtesy of http://www.ndt-ed.org/EducationResources/CommunityCollege/PenetrantTest/PTMaterials/surfaceenergy.htm)

Figure 3.7 Laboratory microscope fitted with a prism for contact angle measurements
The contact angle for a substrate can be measured using the below equation:

$$\gamma_{SV} = \gamma_{LV} \cos \theta + \gamma_{SL}$$

where, \( \gamma_{SV}, \gamma_{LV}, \gamma_{SL} \) = interfacial energies of the solid / vapor, liquid / vapor, solid / liquid interfaces

\( \theta = \) contact angle

(3.1)

3.4.2 Surface Energy

The surface energy or surface tension at the liquid-solid interface is the measure of forces of attraction at the liquid-solid interface. The surface tension study was carried out according to the ASTM standard D2578-84 (Surface Tension test). The surface energy test was carried out using commercially available Dyne Pens (UV process supply, INC, Chicago, IL). A dyne pen is basically a pen filled with a fluid having a particular surface energy. The dyne pens come in sets where the pens have dyne levels or surface energy levels ranging from 30-68 dynes/cm. In order to determine the surface energy, the polymer specimen is placed on a level surface and then the lowest level pen is selected. The tip of the pen is firmly pressed against the surface of the specimen, and a thin line is drawn with the ink from the pen. If the ink line breaks into droplets within 2 seconds then the surface has a surface energy similar or less than the ink, but if the liquid does not break into droplets within 2 seconds then the same procedure is repeated with a higher level dyne pen. Hence, the procedure is repeated with the next higher-level dyne pen till the ink line on the surface breaks into droplets within 2 seconds. When the surface shows beading of the ink, the value of the surface energy of the ink pen used is accepted as the surface energy of the specimen. This procedure is followed at five different sites on the specimen.
3.4.3 O/C ratio

The surface elemental composition study was performed to determine the Oxygen (O), Carbon (C), Phosphorous (P), and Sulfur (S) percentage compositions in Quatromer as well as the six different phospholipid modified Quatromers. The study was carried out using a JSM-5900-LV scanning electron microscope (Jeol, Peabody, MA). Due to limitations with the SEM-EDS system such as calibration for elemental carbon, the study was initially planned to be carried out using an ESCA (Energy Spectroscopy for Chemical Analysis) system, however due to some technical issues with ESCA, the study had to be performed using the SEM-EDS system.

Each of the specimens was prepared in form of small round circles of 10 mm diameter and was placed in the SEM chamber. Once, the samples are placed in the chamber, the SEM scans the surface of the sample using an electron beam. As, the electron beam moves over the sample surface it generates X-ray fluorescence from the atoms in its path. The energy of each of the X-ray photon is collected using the EDS (Energy Dispersive X-ray Spectroscopy) microanalysis system. Each element is said to have a characteristic X-ray photon energy and thus, the EDS system plots the X-rays.
according to their energy and identifies the elements responsible for the peaks in the energy distribution. The data output shows characteristic peaks for each element present on the surface of the sample.

Figure 3.9 JSM-5900-LV scanning electron microscope for surface analysis of specimens (Image courtesy of http://www.fiu.edu/~emlab/inst_SEM.html)

3.5 Comparative Thrombogenic study

The comparative thrombogenic test or the platelet adhesion study was performed to determine the effect of material, flow, and time on platelet deposition. The studies were carried out using Quatromer, PC, EPC, DOPC, DDPC, DAPC, DMPC modified Quatromers, and Sylgard (Dow Corning, Midland, MI) as control. Human blood labeled with Mepacrine dye (Appendix B) having fluorophore-attached platelets was used as the working fluid. A parallel plate flow chamber (Appendix D) was fabricated to allow for a two-dimensional study of blood flow over a test sample and a control, under physiologic flow conditions. The components of the flow chamber included a Lexan top plate, which allows for blood flow, and an aluminum bottom plate embedded with the specimen. The top Lexan plate has a blood flow channel with dimensions of 0.5mm x 5mm x 71mm
which is connected by two inlet and two outlet openings having a diameter of 0.0625 inches. The bottom plate is fabricated from aluminum for added support. The bottom aluminum plate had a rectangular cavity in the center having dimensions of 0.5mm x 25mm x 75mm which allowed a glass slide to be fitted in it. The test sample and the control i.e. sylgard were cured on the glass slide. First the polymer was cured on one third of the slide and then the sylgard was allowed to dry on the remaining two thirds of the same glass slide. A polymer thickness of about 0.3 mm was achieved by pouring 3.8 grams on the glass slide and then 0.75 grams of sylgard was poured on the remaining portion of the slide to achieve a matching thickness of 0.3 mm. The sylgard was allowed to cure on the bottom plate for 24 hours. Once the bottom plate was ready, a thin layer of sylgard was applied around the blood flow channel on the top plate to prevent any leakage of blood. The top and bottom plates were then coupled together using 14 #4-40 flat head screws. The sylgard in the chamber was then allowed to cure for another 24 hours. The sylgard on the top Lexan plate was allowed to cure in the closed chamber to render it air tight thereby preventing any leakage of blood during the experiments. The chamber was placed on the fluorescent microscope stage in such a way that the sylgard on the glass slide was close to the inlet of the chamber while the polymer was towards the outlet. The chamber was placed in such a way in order to ensure that a fully developed laminar flow was flowing over the polymer layer and the latter portion of the sylgard.

Two thin colored paper strips of 10 mm were attached to the outer surface of the glass slide on the sylgard and the polymer portion to act as markers for taking readings at the same points. Six sequences were recorded at the four ends as well as at the center in between the endpoints of the colored strip for sylgard and the polymer. Three steady flow
rates of 10 s\(^{-1}\) (low), 100 s\(^{-1}\) (medium), and 1000 s\(^{-1}\) (high) and two pulsatile flow rates of 100 s\(^{-1}\) (medium), and 1000 s\(^{-1}\) (high) were used in the experiment.

For each experiment, 150mL of human blood was collected into three syringes. The blood was then transferred to a plastic bottle covered by an aluminum foil. The blood was mixed with 150\(\mu\)L of Mepacrine dye and then the bottle was gently inverted few times to mix the platelets with the Mepacrine dye (Appendix C). The blood was divided into eight equal parts to run each of the eight chambers having the Quatromer, the six phospholipid modified Quatromers, and Sylgard. Each of the chambers was run for a period of 30 minutes and pictures were obtained at 5, 15 and 25 minutes for the sample and the control. The samples were photographed using a Nikon Eclipse TS-100 Fluorescence microscopy (Nikon Inc, Melville, NY) and the frames were averaged at a particular time using the Image Pro Plus 4.5 software (Media Cybernetics Inc, CA).

![Figure 3.10 Blood flow in the top plate and the arrangement of specimen on the bottom plate](image)

Figure 3.10 Blood flow in the top plate and the arrangement of specimen on the bottom plate

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![Figure 3.11 Structure of Mepacrine](image)

Figure 3.11 Structure of Mepacrine
3.5.1 Laminar Flow Calculations

In order to minimize the platelet activation in the flow loop and obtain a fully developed laminar flow over the test specimen, the equations below were used to calculate the various dimensions of the flow chamber and the tubing. The equations were based on a flow between two infinite parallel plates.

\[
\frac{Q}{l} = -\frac{1}{12\mu} \left( \frac{\partial P}{\partial x} \right) a^3
\]

where, \( Q = \text{Volume flow rate, } l = \text{unit depth} \) \hspace{1cm} (3.2)

The shear rate was given as:

\[
\frac{du}{dy} = \frac{a^2}{2\mu} \left( \frac{\partial P}{\partial x} \right) \left[ 2y - \frac{1}{a} \right]
\]

where, \( \frac{du}{dy} = \text{shear rate, } l = \text{width of chamber, } a = \text{height of chamber} \) \hspace{1cm} (3.3)

As the shear rate at the walls is zero, \( y = 0 \); hence substituting equation 1 in equation 2 gives

\[
\frac{du}{dy} = \frac{Q}{l} \left( \frac{6}{a} \right) \left[ \frac{1}{a} - \frac{2y}{a^2} \right]
\]

The equation for the entrance length developed by Fox is as followed:

\[
\frac{L}{D} = 0.06R = 0.06 \frac{\rho V D}{\mu}
\]

where, \( V = \frac{Q}{A} = \text{average velocity, } \mu = \text{blood viscosity, } \rho = \text{blood density, and} \) \hspace{1cm} (3.5)

\( D = 4 \frac{A}{P} = \text{hydraulic diameter} \)

The above equation shows that the maximum entrance length is proportional to the flow rate, which in turn is directly proportional to the shear rate. Hence, the maximum
entrance length calculated for the high shear rate (1000 s$^{-1}$) i.e. 1.43 mm was used in the construction of the flow chambers.

The shear rate within the tubing was calculated in order to verify that the tubing had a lower shear rate than the chamber. The following set of equations was used to calculate the shear rate within the tubing.

\[
V = \frac{Q}{\pi r^2} = -\frac{r^2}{8\mu} \left( \frac{\partial P}{\partial x} \right)
\]

where, \(V = \text{average velocity}\)

The point at which the maximum velocity is achieved is calculated as:

\[
\frac{du}{dr} = \frac{1}{2\mu} \left( \frac{\partial P}{\partial x} \right) r
\]

Hence, substituting equation 3 in equation 4 gives the wall shear rate as:

\[
\frac{du}{dr} = -\frac{4V}{r}
\]

The shear rate for the tubing was found out to be 518 s$^{-1}$, which is half of that in the flow chamber i.e. 1000 s$^{-1}$. The lower shear rate in the tube helped ensure minimum platelet activation in the flow loop.

3.5.2 Human Subject Participation and Blood Withdrawal

For each experiment, healthy, normal subjects who did not take any alcohol, aspirin, and Vitamin E were selected to donate blood. The blood was drawn by a licensed phlebotomist using venipuncture. A blood flyer was used to attract the donors (Appendix E). An IRB (Institutional Review Board) approval from the university was obtained, and
the blood was drawn according to the guidelines laid by the IRB. All the participants were required to sign an informed consent before drawing the blood (Appendix E).

For each experiment, 150 mL of human blood was drawn in three 60 mL syringes. Each of the three syringes was first filled with 5 mL of heparin solution. The syringes were then mixed with blood to collect 50 mL blood in all of them. The blood from the three syringes was then emptied into a plastic bottle.

3.5.3 Blood Flow System

A closed flow loop connected using silicone rubber tubing (Cole Parmer, Vernon Hills, IL) was prepared to run blood through the chamber (Figure 3.13). A multi pass 30 min experiment was run to determine the effect of time on the deposition of platelets. The fluorescence labeled blood was held in a reservoir, which was connected to a pump using the silicone tubing. A function generator attached to the peristaltic pump (Cole Parmer, Vernon Hills, IL) helped in pushing the blood through the loop at the required flow rate and a frequency of 1 Hz for pulsatile flow rates. The generator was not used for the steady flow rates. For the steady flow experiments, a removable capacitance chamber was connected to the pump to dampen out the oscillations in the flow. After passing the capacitance chamber, the blood enters the chamber and then returns back to the reservoir. The blood returned to the reservoir is recirculated in loop for 30 minutes (Figure 3.14).
Figure 3.12 Blood Flow Loop for Comparative Platelet Adhesion tests

Figure 3.13 Flow chamber to run blood through the specimen surface

The flow chamber embedded with the sample is placed on the fluorescence microscopy stage (Figure 3.14) and Mepacrine labeled blood is run through the chamber. For these experiments, the fluorescence microscope Nikon Eclipse TS-100 Fluorescence microscopy (Nikon Inc, Melville, NY) lens is connected with an AC UV light source.
The UV light source helps illuminate the Mepacrine labeled platelets thereby allowing us to see the platelet deposition on the polymer surface. The fluorescence microscope is connected to the Streampix software (Norpix Inc, Montreal, Canada), which helps in capturing real time platelet deposition sequences in Norpix Sequence File format. The sequences are then converted to individual TIFF images for a particular time. The TIFF images are then opened using the Image Pro Plus 4.5 software (Media Cybernetics Inc, CA).

Figure 3.14 Flow loop setup with the flow chamber and Nikon Fluorescent microscope

3.5.4 Data Collection and Analysis

Norpix sequences acquired using the Streampix software for each sample at a particular time and for a particular shear rate are first converted to TIFF images. The TIFF images are then averaged using the Image Pro Plus software. First of all, nine focused frames were selected from a sequence recorded at location one (first end-point of the colored strip attached on the outer surface of the glass slide) at a particular shear rate
and $5 \pm 1$ minutes for a specimen. The nine frames were averaged or integrated to obtain one 9-averaged frame at the first location and at time $5 \pm 1$ min. The same procedure was repeated to obtain a single 9-averaged frame for the Sylgard (control) at the first location point and $5 \pm 1$ min time interval. The procedure was repeated to obtain average frames at ten different locations surrounding the colored strip for the specimen as well as the control for the first time interval of $5 \pm 1$ minutes for a particular flow rate. The above procedure was then repeated to obtain ten average frames for both the specimen and Sylgard at two other time intervals of $15 \pm 1$ min and $25 \pm 1$ min respectively at the same flow rate. The above procedure was used to obtain ten average frames for Quatromer, Sylgard, and the six different modified Quatromers at five different flow rates. For example, Figure 3.15 shows a single 9-averaged frame obtained using the ImagePro software where nine focused frames for Quatromer at time $5 \pm 1$ minutes were averaged. The averaging of the frames was required to normalize the errors caused by the AC light source. The AC light source causes fluctuation in light intensity in each frame and hence, there is different light intensity for each of the nine focused frames. Thus in order to normalize the light intensity in each of the nine focused frames, averaging or integration of the frames is carried out to obtain one normalized average frame for each sample at a particular location. The averaging or integration of images is performed to reduce the noise in the image. It has also been found that the presence of AC source results in more brightness in the center of the frame while the corners are darker. Thus, integrating all the nine individual frames helps in obtaining a single integrated frame, which has normalized intensity level throughout the frame. After, the averaging of frames is performed; the average frames for each interval were obtained and inputted in a MATLAB program.
(Appendix F). For each interval time, the average frame for the specimen at location one was used as the sample while the corresponding Sylgard frame at location one and same conditions was used as a reference. The output of the program helped to provide the average platelet intensity value for the specimen at location one and at a particular time interval for a specific flow rate. For example, a single 9-averaged frame of Quatromer at time 5±1 min and location one was obtained as the test sample for the MATLAB program while the corresponding Sylgard 9-averaged frame at 5±1 min and location one was obtained as the reference image. The result obtained after running the MATLAB program gave an average platelet intensity value for Quatromer at time 5±1 min and location one. The above steps were repeated to obtain an average platelet intensity value for the Quatromer at the remaining nine locations for time interval 5±1 min at a particular shear rate. The same process was repeated to obtain ten average platelet intensity values for Quatromer at 15±1 min and 25±1 min respectively at a particular shear rate. The process was also repeated to obtain average platelet intensity values for Quatromer at five different steady and pulsatile shear rates. Hence, the above mentioned steps were repeated to obtain ten average platelet intensity values for Sylgard, and the six phospholipid modified Quatromers at three different time intervals and five different shear rates. The ten average values were used to obtain the mean and the standard error values for each specimen at a given time interval and shear rate. One-way ANOVA tests and post hoc Tukey tests were then performed using SPSS software to determine the statistical significance of the results (Appendix G). For the statistical tests, results were considered to be of significance if p ≤0.05.
3.5.5 Microscope and Chamber Cleaning

The lens of the microscope was cleaned with a cotton swab dipped in a commercially available Fisher optical lens cleaning solution before beginning the experiment as well as after the completion of the experiment. After draining the blood from the flow loop, the flow chamber was disassembled. Then the flow chamber and the other accessories such as the reservoir, and the stainless steel parts were cleaned with a solution of bleach, and tap water by soaking them for 30 minutes to disinfect them. Then each of the components was rinsed with soap and tap water to remove any contamination. The components were then scrubbed with ethyl alcohol pad and were then allowed to dry.
CHAPTER IV

RESULTS

4.0 Results

The result section is divided into mechanical test results, surface analysis results, and the platelet adhesion tests. The first section presents the mechanical testing results, which includes tensile test, peeling test, and shear strength test. The second part includes surface analysis results such as contact angle, surface energy, and O/C ratio. The last section of the study presents the comparative platelet adhesion results.

4.1 Sulfonation of Quatromer

The sulfonation of the Quatromer process was employed to identify its potential in increasing the blood compatibility of the Quatromer. The methylene blue dye staining test was carried out to determine if the sulfonation technique helped in successfully creating sulfonate groups on the Quatromer surface. It was confirmed that when the sulfonated polymer was dipped in methylene blue dye and then washed with distilled water, the surface was stained blue, indicating the presence of sulfonate groups on the Quatromer surface. The surface characterization of the sulfonated Quatromer showed that the contact angle decreased from $74.34^\circ \pm 3^\circ$ (untreated Quatromer) to $68.23^\circ \pm 2^\circ$ (sulfonated Quatromer). One of the interesting findings in the above study was that after 3-4 days, the dye test used to prove the presence of sulfonate groups on the surface did not turn out to be positive. It was found that after 3-4 days of exposure of the sulfonated surface to air, the methylene blue dye test did not stain the sulfonated surface blue. This showed that the functional groups either became inactive or the surface reoriented.
The surface characterization of unsulfonated PC modified Quatromer and sulfonated PC modified Quatromer showed that the contact angle was $74\pm3^\circ$ for both. Hence, a phospholipid grafted sulfonated Quatromer showed the same surface results as the one for phospholipid modified unsulfonated Quatromer which could be explained by the bending of hydrocarbon phospholipid chains on the surface.

4.2 Mechanical Tests

The mechanical tests helped evaluate the tensile strength, surface roughness, and tackiness for Quatromer and the six different phospholipid modified polymers.

4.2.1 Tensile Tests

Two different tensile tests were carried out. The first test was performed to determine the comparative tensile strength between the Quatromer and the various different phospholipid modified Quatromers while the second tensile test helped evaluate the change in tensile test with variation in the %phospholipid concentration in the Quatromer. Tensile tests were carried out to obtain stress-strain data for all the materials. The stress vs. strain curve for Quatromer, 3% PC + Quat, 3% EPC + Quat, 3% DDPC + Quat, 3% DOPC + Quat, 3% DMPC + Quat, and 3% DAPC + Quat is shown in Figure 4.1. The Figure 4.1 shows that the Quatromer has higher tensile strength than all the phospholipid modified Quatromers. Hence, it can be observed that the mixing of phospholipid with the Quatromer causes a decrease in the mechanical strength of the Quatromer. The tensile strengths of all the different phospholipid modified polymers were in a close range when compared to the Quatromer.
Figure 4.1 Stress-Strain graph of Quatromer and different phospholipid modified Quatromers

Table 4.1 Young’s modulus (E), Tensile Stress (TS), and Number of specimens tested (N) of Quatromer and six phospholipid modified Quatromers

<table>
<thead>
<tr>
<th>Material</th>
<th>Modulus Of Elasticity (E) (MPa)</th>
<th>Tensile Stress (TS) at Strain ε=0.45 (MPa)</th>
<th>Number of Specimens (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>3.78 ± 0.73</td>
<td>1.09 ± 0.13</td>
<td>7</td>
</tr>
<tr>
<td>PC + Quat</td>
<td>2.34 ± 0.68</td>
<td>0.85 ± 0.12</td>
<td>7</td>
</tr>
<tr>
<td>EPC + Quat</td>
<td>2.11 ± 0.45</td>
<td>0.73 ± 0.08</td>
<td>7</td>
</tr>
<tr>
<td>DDPC + Quat</td>
<td>2.26 ± 0.96</td>
<td>0.86 ± 0.17</td>
<td>7</td>
</tr>
<tr>
<td>DMPC + Quat</td>
<td>1.96 ± 0.73</td>
<td>0.75 ± 0.13</td>
<td>7</td>
</tr>
<tr>
<td>DAPC + Quat</td>
<td>1.76 ± 0.68</td>
<td>0.70 ± 0.12</td>
<td>7</td>
</tr>
<tr>
<td>DOPC + Quat</td>
<td>1.62 ± 0.68</td>
<td>0.67 ± 0.14</td>
<td>7</td>
</tr>
</tbody>
</table>

As shown in Table 4.1, the Quatromer had a tensile stress (TS) value of 1.09 MPa while for the phospholipid modified Quatromers it was in the range of 0.67-0.86 MPa for similar strain. All the different materials used for testing were found to have elongation of more than 0.45mm/mm without any failure. The specimens could not be stretched beyond that value, as the Enduratec machine is not capable of performing the test beyond
that limit. The tensile stress values were obtained at a strain value of $\epsilon = 0.45$ MPa, and hence the tensile stress values could not be represented as the ultimate tensile strength of the samples. The Young’s modulus of elasticity was calculated using the stress strain values at the initial 10% of the strain. The Table 4.1 shows that the Young’s modulus for elasticity of Quatromer was higher than that obtained for the other phospholipid modified Quatromers. The decrease in the elasticity of the Quatromer on mixing phospholipid can possibly be explained by the phenomenon of plasticization, where the cross linked chains disentangled on adding phospholipids, thereby reducing the modulus of elasticity of the Quatromer.

A second tensile test was performed to determine the effect of increase in the percent phospholipid content in the Quatromer. Figure 4.2 shows the stress strain data for Quatromer, PC 3% + Quat, and PC 15% + Quat. It can be observed from the graph that the increase in the phospholipid concentration in the Quatromer leads to a decrease in the tensile strength of the composite. Table 4.2 shows the Young’s modulus, tensile stress, and strain for the Quatromer and 3% as well as 15% phospholipid/Quatromer composites. It can be observed that the modulus of elasticity of the Quatromer is almost 4 times that of 15% PC modified Quatromer while the modulus of elasticity of 3% PC modified Quatromer is more than twice that of 15% PC modified Quatromer. One of the possible explanations for this phenomenon is that at 15% PC concentration in the polymer, a lot of interlocked or crosslinked chains disentangled thereby resulting in a sharp decrease in the elasticity of the polymer.
Figure 4.2 Stress vs. Strain graph for different %phospholipid modified Quatromers

Table 4.2 Young’s modulus (E), Tensile Stress (TS), and Number of specimens tested (N) of Quatromer and different % phospholipid modified Quatromers.

<table>
<thead>
<tr>
<th>Material</th>
<th>Modulus Of Elasticity (E) (MPa)</th>
<th>Tensile Stress (TS) at Strain ε=0.45 (MPa)</th>
<th>Number of Specimens (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>3.78 ± 0.73</td>
<td>1.09 ± 0.13</td>
<td>7</td>
</tr>
<tr>
<td>PC 3% + Quat</td>
<td>2.34 ± 0.68</td>
<td>0.83 ± 0.12</td>
<td>7</td>
</tr>
<tr>
<td>PC 15% + Quat</td>
<td>0.96 ± 0.45</td>
<td>0.42 ± 0.08</td>
<td>7</td>
</tr>
</tbody>
</table>

4.2.2 Peeling Test

The peeling test was performed to determine the tackiness as well as the interfacial adhesion forces for the Quatromer as well as PC modified Quatromer. Table 4.3 shows that the peeling test performed on two untreated Quatromer strips yielded a peel force of 0.00037 N/m. The peeling test for PC modified Quatromer could not be performed as the strips did not stick to each other and hence, there was significantly less
adhesion between the PC modified surfaces. The difficulty of bonding two PC modified surfaces show that they are significantly less tacky when compared with Quatromer alone.

Table 4.3 Peeling force data for Quatromer and 3% Phosphatidylcholine modified Quatromer

<table>
<thead>
<tr>
<th>Material</th>
<th>Peeling Force (N/m)</th>
<th>Number of Specimens (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>0.00037±0.0004</td>
<td>6</td>
</tr>
<tr>
<td>PC 3 % + Quat</td>
<td>-</td>
<td>6</td>
</tr>
</tbody>
</table>

4.2.3 Shear Test

The shear test was also performed to determine the interfacial adhesion force between two layers of Quatromer as well as two layers of PC modified Quatromer. The shear strength for the Quatromer was determined to be 0.00211 N/m while the shear force for the PC modified Quatromer was 0.000545 N/m (Table 4.4). Hence, it can be observed that the interfacial shear force required to separate two Quatromer surfaces is almost four times more than that required to separate two PC modified Quatromer surfaces. Hence, it can be found that PC modification of the Quatromer helps in decreasing the tackiness of the polymer.

Table 4.4 Shear strength data for Quatromer and 3% Phosphatidylcholine modified Quatromer

<table>
<thead>
<tr>
<th>Material</th>
<th>Shear Force (N/m)</th>
<th>Number of Specimens (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>0.00211±0.0004</td>
<td>6</td>
</tr>
<tr>
<td>PC 3 % + Quat</td>
<td>0.000545±0.0005</td>
<td>6</td>
</tr>
</tbody>
</table>
4.3 Surface Analysis

The surface analysis includes the contact angle, surface energy, and O/C ratio measurements.

4.3.1 Contact angle and Surface Energy

The contact angle and surface energy measurements are correlated as they are governed by the same forces of cohesion and adhesion. The decrease in contact angle is directly related to the increase in the surface energy of the surface. Table 4.5 shows that the different phospholipid modification of Quatromer decreases the contact angle as well as increases the surface energy as compared to the untreated Quatromer. The contact angles for PC + Quat, DOPC + Quat, DDPC + Quat, and DMPC + Quat were in the range of 12-18° while that for EPC + Quat and DAPC + Quat were in the range of 31-38°.

Table 4.5 Contact angle and surface energy data for Quatromer and six phospholipid modified Quatromers

<table>
<thead>
<tr>
<th>Material</th>
<th>Contact Angle (Degrees)</th>
<th>Surface Energy (Dynes/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>74.34±3</td>
<td>36-38</td>
</tr>
<tr>
<td>Phosphatidylcholine + Quat</td>
<td>17.12±3</td>
<td>62-64</td>
</tr>
<tr>
<td>Ethanolaminephosphatidylcholine + Quat</td>
<td>37.34±4</td>
<td>52-54</td>
</tr>
<tr>
<td>1,2-Dimyristooyl-rac-glycero-3-phosphocholine + Quat</td>
<td>12.24±3</td>
<td>62-64</td>
</tr>
<tr>
<td>1,2-Didecanoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>13.94±4</td>
<td>62-64</td>
</tr>
<tr>
<td>1,2-Dilinoleoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>16.76±3</td>
<td>62-64</td>
</tr>
<tr>
<td>1,2-Diarachidonoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>31.33±3</td>
<td>56-58</td>
</tr>
</tbody>
</table>

The above results show that the modification of the Quatromer with the phospholipids increases the adhesion forces between the liquid and the solid polymer surface thereby overcoming the cohesion forces within the liquid molecule.
4.3.2 O/C ratio

The oxygen carbon ratio was measured to determine the amount of free oxygen as well as determine the carbonyl groups in the untreated as well as the various phospholipid modified Quatromers. It has been observed by Ertel that an increase in O/C ratio produces higher oxygen content and carbonyl groups on the surface, which correlates with an increase in the number of reactive negatively charged sites and enhanced endothelial cell growth (Ertel 1990). Table 4.6 shows the O/C ratio for the Quatromer and the six different phospholipid modified Quatromers.

Table 4.6 O/C ratio data for Quatromer and six phospholipid modified Quatromers

<table>
<thead>
<tr>
<th>Material</th>
<th>O/C ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quatromer</td>
<td>0.042</td>
</tr>
<tr>
<td>Phosphatidylcholine + Quat</td>
<td>0.061</td>
</tr>
<tr>
<td>Ethanolaminephosphatidylcholine + Quat</td>
<td>0.061</td>
</tr>
<tr>
<td>1,2-Dimyristoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>0.069</td>
</tr>
<tr>
<td>1,2-Didodecanoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>0.062</td>
</tr>
<tr>
<td>1,2-Dilinoleoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>0.064</td>
</tr>
<tr>
<td>1,2-Diarachidonoyl-rac-glycero-3-phosphocholine + Quat</td>
<td>0.061</td>
</tr>
</tbody>
</table>

The above results show that the Quatromer has less oxygen as well as a lower O/C ratio than all of the phospholipid modified Quatromers. Hence, it can be said that the phospholipid modification helps in increasing the carbonyl group concentration in the Quatromer thereby increasing the number of negatively charged groups on the surface. Table 4.6 shows that the Quatromer has an O/C ratio of 0.042, however, it is known that the structure of Quatromer has no oxygen atoms present in it. One of the possible reasons for this phenomenon is that the Quatromer surface might have adsorbed water vapor from the atmosphere and this may have resulted in the presence of oxygen in the Quatromer.
4.4 Comparative Thrombogenic Tests

The comparative thrombogenic tests helped determine the effect of material, flow, and time on platelet adhesion on the untreated Quatromer, the control i.e. Sylgard, and each of the six modified phospholipid Quatromers. The tests were carried out at three different steady flow shear rates of 10 s⁻¹, 100 s⁻¹, and 1000 s⁻¹, and two different pulsatile flow shear rates of 100 s⁻¹, and 1000 s⁻¹.

In order to design a flow chamber and a flow loop, which would result in the minimum platelet activation and laminar flow over the sample, proper tubing and blood flow channel dimensions were calculated. The results from Table 4.7 below gives the radius of the tubing and the minimum entrance length required for the chamber to have laminar flow conditions with minimum platelet activation. The highest flow rate used in the chamber was 12.50 mL/min or a shear rate of 1000 s⁻¹, which required a minimum entrance length of 1.43 mm to generate laminar flow. The Masterflex L/S 14 tubing (Cole-Parmer, Vernon Hills, IL) was selected as it generates a shear rate (518 s⁻¹) that is almost half of that present in the flow chamber. This low shear rate in the tubing helps to reduce the platelet activation.

Table 4.7 Laminar flow calculations for the flow chamber and tubing

<table>
<thead>
<tr>
<th>Flow rate (mL/min)</th>
<th>du/dy_{chamber} (s⁻¹)</th>
<th>R_{chamber}</th>
<th>V_{chamber} (cm/sec)</th>
<th>L_{chamber} (mm)</th>
<th>du/dr_{tube} (s⁻¹)</th>
<th>r_{tube}</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.21</td>
<td>16.80</td>
<td>0.40</td>
<td>0.14</td>
<td>0.02</td>
<td>8.70</td>
<td>0.80</td>
</tr>
<tr>
<td>1.25</td>
<td>100.00</td>
<td>2.38</td>
<td>0.83</td>
<td>0.14</td>
<td>51.80</td>
<td>4.74</td>
</tr>
<tr>
<td>12.50</td>
<td>1000.00</td>
<td>23.80</td>
<td>8.33</td>
<td>1.43</td>
<td>518.00</td>
<td>47.37</td>
</tr>
</tbody>
</table>

Figures 4.3 and 4.4 below show Mepacrine labeled platelet deposition pictures for Quatromer and DMPC modified Quatromer at 5±1 and 25±1 minutes respectively. It can
be observed from figures 4.3 and 4.4 that the Quatromer has much higher platelet deposition at times min and 25±1 min when compared to DMPC modified Quatromer at the same times.

Figure 4.3 Fluorescent microscopy pictures of Quatromer surface at 5 min (left) and 25 min (right)

Figure 4.4 Fluorescent microscopy pictures of DMPC modified Quatromer surface at 5 min (left) and 25 min (right)

The average platelet intensity values obtained from the Matlab program are plotted in the tables and figures below to show that the effect of material, shear rate, and time on platelet adhesion and activation on the surface. The platelet deposition experiments were performed for the control i.e. Sylgard, unmodified Quatromer, and six different phospholipid modified Quatromers namely, PC + Quatromer, EPC + Quatromer, DDPC + Quatromer, DMPC + Quatromer, DOPC + Quatromer, and DAPC + Quatromer. Five
different shear rates i.e. steady low (10 s⁻¹), steady medium (100 s⁻¹), and steady high (1000 s⁻¹), pulsatile medium (100 s⁻¹), and pulsatile high (100 s⁻¹) and three different time periods i.e. 5±1 min, 15±1 min, 25±1 min were used to evaluate the samples.

Tables 4.8, 4.9, 4.10 and Figures 4.5, 4.6, 4.7 shows the platelet intensity data at low, medium, and high steady shear rates, while Tables 4.11, 4.12 and Figures 4.9, 4.9 shows the platelet intensity at medium and high pulsatile shear rates. The results at all shear rates show that the platelet intensity is the highest for the Quatromer while the control i.e. Sylgard also had a high platelet deposition on the surface. The platelet intensity data also shows that the Quatromer and the control i.e. Sylgard have much higher platelet deposition than any of the phospholipid modified Quatromers while the DDPC, DMPC, PC, and the DOPC modified Quatromers have lowest platelet deposition. Also, it was observed that the platelet deposition increased for all specimens with time. It was found that the platelet deposition was significantly high especially for Quatromer and Sylgard at the high (1000 s⁻¹) steady and pulsatile shear rates when compared to low and medium shear rates.

Tables 4.13, 4.14, 4.15 and Figures 4.10, 4.11, 4.12 shows the platelet density at 5±1 min, 15±1 min, and 25±1 min respectively for Sylgard, Quatromer and different modified Quatromers at different steady and pulsatile shear rates. The comparison carried out in the above figures show that the platelet deposition at steady and pulsatile shear rates did not demonstrate any significant differences. Tables F1, F2, F3, F4, F5, and F6 (Appendix G) show the One-way ANOVA and post hoc tests for all the samples at different shear rates. One-way ANOVA and post hoc Tukey tests (Appendix G) performed showed that
the PC, DMPC, and DDPC modified Quatromers had the lowest platelet adhesion with no significant difference for different shear rates.

Table 4.8 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady low shear rate

<table>
<thead>
<tr>
<th>Material</th>
<th>Time interval</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5±1 min</td>
</tr>
<tr>
<td>Slygard</td>
<td>15.44±1.21</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>19.71±1.95</td>
</tr>
<tr>
<td>PC+Q</td>
<td>3.16±1.52</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>8.52±1.37</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>2.44±1.12</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>2.49±1.51</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>5.12±1.24</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>7.24±1.61</td>
</tr>
</tbody>
</table>

Figure 4.5 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady low shear rate
Table 4.9 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady medium shear rate

<table>
<thead>
<tr>
<th>Material</th>
<th>5±1 min</th>
<th>15±1 min</th>
<th>25±1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slygard</td>
<td>13.38±1.92</td>
<td>19.73±2.84</td>
<td>39.58±2.41</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>23.43±1.48</td>
<td>28.34±4.17</td>
<td>42.13±3.16</td>
</tr>
<tr>
<td>PC+Q</td>
<td>4.77±1.01</td>
<td>6.64±2.32</td>
<td>7.14±1.94</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>8.67±1.16</td>
<td>10.99±1.66</td>
<td>14.32±1.87</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>3.13±1.25</td>
<td>4.74±0.98</td>
<td>6.31±1.27</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>4.23±1.41</td>
<td>6.61±1.34</td>
<td>7.62±1.84</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>4.92±1.53</td>
<td>5.52±2.23</td>
<td>6.72±1.43</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>7.39±2.22</td>
<td>10.80±1.72</td>
<td>12.52±2.85</td>
</tr>
</tbody>
</table>

Figure 4.6 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady medium shear rate
Table 4.10 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady high shear rate

<table>
<thead>
<tr>
<th>Material</th>
<th>5±1 min</th>
<th>15±1 min</th>
<th>25±1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sylgard</td>
<td>19.42±2.90</td>
<td>36.73±2.13</td>
<td>53.99±1.33</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>30.43±3.45</td>
<td>40.28±2.84</td>
<td>53.18±1.64</td>
</tr>
<tr>
<td>PC+Q</td>
<td>5.97±1.72</td>
<td>7.34±1.32</td>
<td>7.83±1.45</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>8.28±1.17</td>
<td>10.87±1.54</td>
<td>12.73±1.52</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>2.84±0.72</td>
<td>3.51±1.32</td>
<td>4.66±1.29</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>3.15±1.19</td>
<td>4.78±1.57</td>
<td>5.12±1.35</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>3.12±1.04</td>
<td>4.52±1.69</td>
<td>6.98±2.39</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>9.27±1.47</td>
<td>15.80±2.98</td>
<td>18.52±1.92</td>
</tr>
</tbody>
</table>

Figure 4.7 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Steady high shear rate
Table 4.11 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Pulsatile medium shear rate

<table>
<thead>
<tr>
<th>Material</th>
<th>5±1 min</th>
<th>15±1 min</th>
<th>25±1 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slygard</td>
<td>12.21±1.93</td>
<td>24.73±3.35</td>
<td>37.17±2.92</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>21.92±1.26</td>
<td>31.22±2.54</td>
<td>39.67±3.55</td>
</tr>
<tr>
<td>PC+Q</td>
<td>4.16±1.21</td>
<td>4.83±1.46</td>
<td>6.15±1.29</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>7.58±1.16</td>
<td>8.98±2.26</td>
<td>10.12±1.35</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>4.34±0.63</td>
<td>4.89±1.34</td>
<td>5.67±1.31</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>3.18±1.11</td>
<td>4.61±1.65</td>
<td>5.23±1.43</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>5.52±1.77</td>
<td>6.53±1.52</td>
<td>7.18±3.03</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>7.78±1.21</td>
<td>9.80±2.23</td>
<td>11.61±1.96</td>
</tr>
</tbody>
</table>

Figure 4.8 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Pulsatile medium shear rate
Table 4.12 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at Pulsatile high shear rate

<table>
<thead>
<tr>
<th>Material</th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
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</tr>
<tr>
<td>Slygard</td>
<td>22.14±2.21</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>27.05±1.95</td>
</tr>
<tr>
<td>PC+Q</td>
<td>5.68±1.52</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>8.59±1.77</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>2.76±1.52</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>2.88±1.04</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>5.14±1.34</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>10.24±1.81</td>
</tr>
</tbody>
</table>

Figure 4.9 Platelet Intensity (Pl) for Pulsatile High Shear rate 100s⁻¹
Table 4.13 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 5±1 min time interval

<table>
<thead>
<tr>
<th>Material</th>
<th>LS</th>
<th>MS</th>
<th>HS</th>
<th>MP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sylgard</td>
<td>15.44±1.21</td>
<td>13.38±1.92</td>
<td>19.42±2.90</td>
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<td>22.14±2.21</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>19.71±1.95</td>
<td>23.43±1.48</td>
<td>30.43±3.45</td>
<td>21.92±1.26</td>
<td>27.05±1.95</td>
</tr>
<tr>
<td>PC+Q</td>
<td>3.16±1.52</td>
<td>4.77±1.01</td>
<td>5.97±1.72</td>
<td>4.16±1.21</td>
<td>5.68±1.52</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>8.52±1.37</td>
<td>8.67±1.16</td>
<td>8.28±1.17</td>
<td>7.58±1.16</td>
<td>8.59±1.77</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>2.44±1.12</td>
<td>3.13±1.25</td>
<td>2.84±0.72</td>
<td>4.34±0.63</td>
<td>2.76±1.52</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>2.49±1.51</td>
<td>4.23±1.41</td>
<td>3.15±1.19</td>
<td>3.18±1.11</td>
<td>2.88±1.04</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>5.12±1.24</td>
<td>4.92±1.53</td>
<td>3.12±1.04</td>
<td>5.52±1.77</td>
<td>5.14±1.34</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>7.24±1.61</td>
<td>7.39±2.22</td>
<td>9.27±1.47</td>
<td>7.78±1.21</td>
<td>10.24±1.81</td>
</tr>
</tbody>
</table>

Figure 4.10 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 5±1 min time interval
Table 4.14 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 15±1 min time interval

<table>
<thead>
<tr>
<th>Material</th>
<th>Shear rate s⁻¹</th>
<th>LS</th>
<th>MS</th>
<th>HS</th>
<th>MP</th>
<th>HP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Slygard</td>
<td></td>
<td>21.73±2.35</td>
<td>19.73±2.84</td>
<td>36.73±2.13</td>
<td>24.73±3.35</td>
<td>39.73±2.55</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td></td>
<td>30.30±3.55</td>
<td>28.34±4.17</td>
<td>40.28±2.84</td>
<td>31.22±2.54</td>
<td>42.54±3.85</td>
</tr>
<tr>
<td>PC+Q</td>
<td></td>
<td>4.33±1.12</td>
<td>6.64±2.32</td>
<td>7.34±1.32</td>
<td>4.83±1.46</td>
<td>6.33±1.32</td>
</tr>
<tr>
<td>EPC+Q</td>
<td></td>
<td>11.72±1.71</td>
<td>10.99±1.66</td>
<td>10.87±1.54</td>
<td>8.98±2.26</td>
<td>14.22±2.66</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td></td>
<td>4.11±1.82</td>
<td>4.74±0.98</td>
<td>3.51±1.32</td>
<td>4.89±1.34</td>
<td>5.31±1.32</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td></td>
<td>3.78±1.75</td>
<td>6.61±1.34</td>
<td>4.78±1.57</td>
<td>4.61±1.65</td>
<td>3.82±1.07</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td></td>
<td>6.78±1.51</td>
<td>5.52±2.23</td>
<td>4.52±1.69</td>
<td>6.53±1.52</td>
<td>7.37±2.02</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td></td>
<td>9.21±1.85</td>
<td>10.80±1.72</td>
<td>15.80±2.98</td>
<td>9.80±2.23</td>
<td>14.61±2.44</td>
</tr>
</tbody>
</table>

Figure 4.11 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 15±1 min time interval
Table 4.15 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 25±1 min time interval

<table>
<thead>
<tr>
<th>Material</th>
<th>Shear rate s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LS</td>
</tr>
<tr>
<td>Slygard</td>
<td>41.13±3.73</td>
</tr>
<tr>
<td>Quat (Q)</td>
<td>42.68±2.77</td>
</tr>
<tr>
<td>PC+Q</td>
<td>6.24±1.95</td>
</tr>
<tr>
<td>EPC+Q</td>
<td>13.34±1.92</td>
</tr>
<tr>
<td>DMPC+Q</td>
<td>4.77±1.39</td>
</tr>
<tr>
<td>DDPC+Q</td>
<td>4.43±1.15</td>
</tr>
<tr>
<td>DOPC+Q</td>
<td>7.58±2.33</td>
</tr>
<tr>
<td>DAPC+Q</td>
<td>10.72±2.36</td>
</tr>
</tbody>
</table>

Figure 4.12 Platelet Intensity for Sylgard (control), Quatromer and different phospholipid modified Quatromers at different shear rates for 25±1 min time interval
5.0 Discussions

5.1 Is Sulfonation of Quatromer required?

The sulfonation of Quatromer was employed to identify its role in enhancing the Quatromer's material properties. It is known that the Quatromer is highly stable and does not have any free functional groups on the surface and hence, there are no surface charges on the Quatromer surface. Hence, the sulfonation of Quatromer was employed to generate functional groups such as $\text{SO}_3^{-}$ on the surface, which may help in rendering surface charges to the Quatromer surface.

The results showed that after 2-3 days of exposure of the sulfonated Quatromer surface with air, the methylene blue dye test was negative. One of the explanations for this occurrence was that the sulfonate groups on the surface became inverted or reoriented towards the inside when exposed to the air. It has been observed that functional groups such as sulfonates tend to show a pattern of reversibility when exposed to air (Figure 5.1). The sulfonate groups tend to move towards the interior of the Quatromer due to surface tension. This reorientation of the functional groups shows the aging of the surface modification process. However, when the sulfonated Quatromer is drenched in water, the functional groups tend to reorient towards the outside.
Figure 5.1 Reversibility or aging of sulfonate functional groups in presence of air

Also, Quatromer modified with just phospholipid and Quatromer modified using both sulfonation process as well as phospholipid had a similar contact angle of \(74\pm3^\circ\), thereby showing the same surface analysis results. One of the possible reasons for this phenomenon was that the phospholipid attached to the sulfonate groups had a bending of their hydrophobic chains (Figure 5.2).

Even though results from the surface characterization of sulfonated Quatromer showed that the contact angle or the surface properties did not have significant improvements when compared to untreated Quatromer, yet the grafting of sulfonate groups would play an important role in improving the Quatromer’s implant properties. The sulfonation of Quatromer would help reduce the tackiness of the Quatromer which is desired in manufacturing of the valve, and would help form possible cross linkages to reinforce the strength of the Quatromer. The sulfonation process would help in rendering a homogenous surface as well as help to impart antimicrobial activity to the Quatromer.
surface, which may not be achieved just using phospholipid modification (Hee et al. 2000).

Figure 5.2 Sulfonation of Quatromer and phospholipid grafting on sulfonated Quatromer

5.2 Surface modification vs. phase mixing modification of Quatromer

Two types of modification processes were used to attach phospholipid to the Quatromer surface. The first method was surface modification, which was achieved by dipping the untreated Quatromer surfaces in 20% phospholipid/Quatromer solution. The other method, which was more of structural modification, involved mixing of 3% phospholipid to the Quatromer by weight. Surface modification of Quatromer using dip coating is an easy, relatively inexpensive, and quick method; however it suffers from many disadvantages when compared to phase mixing of phospholipid with the
Quatromer. One of the major disadvantages of the dipping process was the lack of strong attachment of the phospholipid groups to the Quatromer surface. Preliminary experiments carried out in the Cardiovascular Engineering lab (CVEC, Florida International University) showed that when a Quatromer substrate was dipped in additional Quatromer solution, peeling occurred between the new layer formed and the Quatromer substrate. This showed that the dip coating lacked the ability to allow stronger attachment of the surfactant layer onto the Quatromer surface. This drawback of the dipping process would result in a very small layer of phospholipids on the Quatromer surface. The small superficial layer of phospholipid formed on the Quatromer surface may limit or interfere with the self-assembling of the phospholipids into bilayers. The absence of the bilayers in the Quatromer may detrimentally affect the blood compatibility properties of the Quatromer, as the highly ordered bilayers as shown in this thesis enhance the hemocompatibility of the polymer. Studies performed by van der Heiden using DOPC on polyurethanes showed that the presence of mere phosphatidylcholine groups on the polyurethane surface was not sufficient to suppress protein adsorption, rather the highly ordered structure of phospholipid bilayers is much more effective in suppressing the protein adsorption (van der Heiden AP et al, 1998). In the case of a phase mixture of a 3% phospholipid/Quatromer by weight, the phospholipids are believed to be distributed homogeneously throughout the Quatromer material and the phospholipid has the ability to self assemble to form bilayers inside the polymer body (Figure 5.3). Another drawback of surface modification is that the layer of phospholipid on Quatromer may have a limited amount of phospholipid, which may detach or suffer oxidation reactions forming free radicals or peroxides with time. However, in a 3% phase phospholipid/Quatromer
mixture, the phospholipids would keep migrating towards the surface by the means of passive diffusion, replacing those on the surface that may become detached or are destroyed (Figure 5.3). Thus, in an implantable device such as a heart valve, which is in human body for 7-10 years, the phospholipid/polymer phase mixture would be advantageous, as there would be constant migration of phospholipids towards the surface providing the polymer with a longer lasting hemocompatible surface.

Figure 5.3 Phospholipid orientation in phase mixed Quatromer (top) and a surface modified Quatromer (bottom)

Another major drawback faced by traditional dip coating method is that the surfactant distribution on the polymer surface would be difficult to control, i.e., it may not be uniform and homogeneous. Traditional dip coating process causes a “wedge effect” i.e. the film thickness varies from top to the bottom as well as entraps bubbles while curing the surface. For example, when Quatromer leaflets were fabricated in the Cardiovascular Engineering lab (CVEC, Florida International University) by dipping the
mold in a 15% Quatromer solution, the leaflets tend to show a lot of bubbles trapped inside them. Thus, dip coating process may result in sites on the Quatromer surface where the PC coating is thin or absent. These sites may increase the potential of platelet activation and adhesion. The failure to modify the surface uniformly may also result in increased surface roughness. In contrast, a 3% phospholipid/Quatromer phase mixture has sufficient phospholipid to distribute it homogeneously and uniformly within the bulk material as well as on the surface. The mixing would also help in creating a much smoother Quatromer surface.

5.3 Effect of phospholipid acyl chain unsaturation and length

The chain length and degree of unsaturation of the acyl or hydrocarbon chains in phospholipid has a vital relationship with hydrophobicity of the surface and biological effects. It has been found that saturated phospholipids tend to be less hydrophobic in nature while the unsaturation of phospholipids tends to decrease hydrophilicity or decrease water penetration from the polar group region (Subczynski 1994). The double bond results in a kink in the hydrocarbon tail (Figure 5.4), which results in a disorder conformation of the acyl chain. The kink in the tail prevents compact arrangement of the phospholipids thereby decreasing the water penetration around the polar head group. It has been observed that longer acyl chain lengths tend to increase the width of the central hydrophobic regions of the hydrocarbon chains (Subczynski 1994). The increase in the chain length is a minor contributor for the increased hydrophicity while the introduction of double bond in the chain is the major contributor. Moreover, it has been found that the
hydrophobicity effect is more dominant due to the presence of a cis double bond rather than a trans double bond (Subczynski 1994).

Experiments carried out by Akira Tokumura and group on Lysophosphatidic acid (LPAs) showed a rank order for platelet aggregation potential for various saturated and unsaturated LPAs having different chain lengths. The order for aggregating activity of LPAs was $16:0 > 18:2 > 18:3 > 18:1 > 18:0 > 14:0 > 12:0$ where the first digit represents the number of carbon atoms while the second digit indicates the number of double bonds in acyl chain (Tokumura 2002). The order shows that the shortest and saturated phospholipids have the lowest platelet aggregating activity. This may be one of the reasons for the lowest platelet intensity obtained for DMPC+Quat and DDPC+Quat specimens. The DMPC and DDPC phospholipids used to modify the Quatromer have a structure of 14:0 and 12:0 respectively. As observed in figure 5.4, unsaturated phospholipids have a kink in the tail and this resulted in spaces in the bilayer. The presence of these spaces results in increased protein associations, while a saturated phospholipid has fatty acids sticking closer to each other thereby preventing any empty spaces within the bilayer. Hence, unsaturation of the phospholipid hydrocarbon chains may be a contributing factor in increased platelet adhesion.

![Figure 5.4 Bilayer arrangements of a saturated (left) and an unsaturated (right) phospholipid](image-url)

Figure 5.4 Bilayer arrangements of a saturated (left) and an unsaturated (right) phospholipid
One of the important parts of this research was to determine the oxidation damage of phospholipids due to aging. The acyl chain unsaturation has an important relationship with the oxidation of the double bonds resulting in the formation of peroxides. The CH bond in an unsaturated phospholipid is susceptible to spontaneous oxidation resulting in formation of free radicals such as peroxides (Seelig 1977). These radicals are highly unstable and they tend to increase the oxidation stress in the materials. Hence, highly unsaturated phospholipids such as DAPC, which have arachidonate group having four double bonds is highly susceptible to oxidation in the body and they may be destroyed faster in vivo. Saturated phospholipids such as DDPC and DMPC that have no double bonds in their hydrocarbon chains and hence, suffer from less oxidation reactions due to the presence of a stable and ordered hydrocarbon tail structure. Also the addition of ethanolamine to phospholipids has shown to decrease the oxidizability of CH bonds (Maeba 2003). Hence, EPC+Q may help increase the oxidation resistance of the CH bonds in the Quatromer. Most of the unsaturated phospholipids have a cis double bond. The cis double bonds are responsible for producing the kink in the hydrocarbon tail and hence results in a loosely packed bilayer. This bilayer arrangement with spaces in it results in greater oxidation of the CH bonds. In contrast, the presence of trans double bond results in absence of a kink in the hydrocarbon tail as the hydrogen atoms of the double bond are on either side of the fatty acid. The absence of the kink in the tail causes them to behave similar to saturated fatty acids even though they are unsaturated. The trans bond results in formation of a compact bilayer where the fatty acids are stuck close to each other and this provides increased oxidation resistance (Elena, 2003). Hence
addition of trans unsaturated phospholipids such as Sphingomyelin may also help increase the oxidation resistance of the Quatromer.

Temperature also plays an important role in affecting the properties of the phospholipids. Studies performed have shown that an increase in unsaturation of the phospholipids tend to lower their phase transition temperature $T_m$ (Huang 1969). The phase transition temperature $T_m$ is the temperature at which the hydrocarbon chains in the phospholipid undergo a change from an ordered state to a disordered state (Huang 1969). In the ordered state the fatty acids in the phospholipids have a compact arrangement and this tends to increase the hydrophilicity of the phospholipids. It has been observed that at low temperatures (below $T_m$) the bilayers in phospholipids tend to have an ordered, and a solid gel-like structure while at high temperatures (above $T_m$) the bilayers exist in a disordered fluid state (Huang 1969).

Unsaturated phospholipids as explained earlier have a kink in their tails, and this bending of the acyl chains is supposed to reduce the melting temperature $T_m$ of the phospholipids as the amount of interaction between the acyl chains decreases and this subsequently increases the Gibbs free energy (Wang 1997). Trans double bonds as compared to cis double bonds tend to increase the melting temperature of the phospholipids as the acyl chains have greater contact between them (Wang 1997). Thus, it can be observed that an increase in the temperature as well as unsaturation in the phospholipids can possibly decrease the stability and the melting temperature of the phospholipids, which in turn may affect its hydrophilic properties as well as structural stability.
5.4 Mechanical Testing

The mechanical testing of the Quatromer and the different PC modified Quatromers was carried out to evaluate the effect of phospholipid modification on the bulk properties of the polymer and the effect on the tackiness as well as the surface texture of the polymer. The tensile test performed on the untreated Quatromer and the six different PC modified Quatromers showed that the addition of phospholipids to the Quatromer resulted in decrease in the tensile strength of the Quatromer. Also, the second tensile test performed on untreated Quatromer and different % phospholipid/Quatromer mixtures showed that the tensile strength decreased with increase in the % phospholipid concentration in the Quatromer. The tensile tests show that the modulus of elasticity of all the six different phospholipid modified Quatromers is almost half of that of untreated Quatromer. One of the possible reasons for the decrease in the modulus of elasticity of the Quatromer on adding a phospholipid is due to the phenomenon of plasticization. The unmodified Quatromer structure has crosslinked or entangled hydrocarbon chains and the addition of phospholipids to Quatromer tends to untangle the crosslinked chains. The straightening or detangling of the hydrocarbon chains, known as plasticization, may result in the decrease of the modulus of elasticity of the phospholipid modified Quatromer. The tests performed using different % phospholipid concentrations in Quatromer implied a direct relationship between an increase in the phospholipid concentration and decrease in elastic modulus. One of the probable reasons of such a huge decrease in the modulus of elasticity at 15% PC modification when compared to 3% concentration is that at such a high PC concentration in the Quatromer, a large amount of
crosslinked hydrocarbon chains may be detangled resulting in greater loss of the modulus of elasticity of the Quatromer.

The peeling and the shear strength tests were performed to determine the effect of phospholipid on the tackiness of the Quatromer. The shear strength test showed that the interfacial force required to separate Quatromer surfaces is four times higher than that required for separating PC modified surfaces attached together. The peeling test could not be performed for PC modified surfaces indicating that the surfaces lacked the tackiness to attach to each other. Hence it can be proved that the tackiness of the Quatromer reduces significantly with addition of phospholipids. The reduced tackiness for PC modified surfaces can be explained by the fact that the PC modification results in a smooth surface. The smooth surface helps reduce the anchorage (micro pits, cracks or material mounts) sites on the surface thereby reducing the tackiness or stickiness of the Quatromer. The PC modification also helps in imparting lubricity or reduced friction to the Quatromer surface as the hydrophilic nature of the phospholipid creates a water interface or layer at the Quatromer surface, thereby reducing wet friction on the surface.

The reduced stickiness of the Quatromer is desired for the ease of manufacturing heart valve leaflets. The Quatromer is sticky and when the Quatromer leaflets are fabricated using the mold, the Quatromer sticks to the mold and this causes empty voids in the leaflets. To peel the Quatromer from the mold, it requires a process of adding baking soda to remove the stickiness of the polymer and thus, modifying the Quatromer with phospholipid helps reduce the stickiness of the Quatromer. The peeling and shear strength tests show that the modified Quatromer is significantly less sticky and thus while
peeling the modified Quatromer leaflets from the mold; the polymer does not stick to the mold thereby causing no voids in the leaflets.

5.5 Surface Analysis

The surface analysis of the Quatromer and PC modified Quatromer surfaces was carried out to determine the hydrophilicity as well as the surface roughness of the surfaces. The contact angle as well as the surface energy measurement studies showed that the PC modification notably increased the surface hydrophilicity of the Quatromer surface. The phosphate head groups tend to loosely bind water molecules on the surface thereby increasing the wettability of the surface. The hydrophilic nature of the PC head group possibly forms a thin water layer on the surface of the Quatromer thereby allowing the body fluids to flow easily over it without activating the components in it.

The O/C carbon results obtained for the research study may not be accurate as they were obtained using the EDS-SEM system. It is known that the EDS-SEM system is not a good tool to determine the elemental composition of substrates, especially for carbon determination due to potential drawbacks such as poor spectral resolution and calibration of carbon. Thus, initially the study was supposed to be performed using an ESCA (Electron Spectroscopy for Chemical Analysis) system at FIU; however, due to some technical problems with the ESCA system, the O/C ratio study had to be performed using the EDS-SEM system.

The O/C ratio was calculated in order to determine the increase in the amount of oxygen as well as carbonyl groups on the Quatromer surface. The PC modification was effective in increasing the O/C content, which increased the negatively charged carbonyl
groups on the surface. These negatively charged carbonyl groups present on the surface may be the main force in repelling the negatively charged platelets from attaching on the Quatromer surface. The carbonyl groups played an important role in enhancing the hydrophilic nature of the Quatromer. The O/C ratio results showed the presence of oxygen in Quatromer; however it is known that Quatromer is composed of only carbon and hydrogen atoms and no oxygen atoms. One of the possible reasons for this phenomenon is that the Quatromer surface may have absorbed water vapor from the atmosphere and the oxygen atoms present in the water vapor may have been responsible for oxygen content observed in Quatromer during the SEM studies.

5.6 Comparative Platelet Studies

The comparative platelet adhesion tests were performed to determine the potential of Quatromer as well as the different phospholipid modified Quatromers in activating and attaching platelets on their surface over a period of time as well as at different shear conditions. A multi-pass flow loop was used to determine the effect of time on the surface properties of the material in activating the platelets. The multi-pass system helped identify if the functional groups on the modified surface tend to show their effect over time or if they suffered from various problems such as oxidation reactions, reorientation of the functional groups or erosion. There were several sources in the experiments, which may result in errors in the results. One of the sources is the use of a multi-pass system, which would suffer from increased platelet activation due to the perturbing effects of the peristaltic pump, yet as the study is a comparative evaluation of all the polymers, there is a negligible impact on the conclusions. Also, the pictures obtained using the fluorescent
microscopy used an AC light source and this resulted in more brightness in the center of
the frame while the edges were dark. Another source, which may cause error, is the
inability of the ImagePro software or the MATLAB program to differentiate between
Mepacrine labeled platelet aggregates and WBCs in the blood. Thus, when the flow loop
was run, both the WBCs as well as the platelets were observed in the frames and there
was no way the WBCs could be differentiated from the platelet aggregates. To overcome
these problems nine best-focused frames were selected at a single time and at the same
conditions for each material. The frames were then averaged using the ImagePro
software. The averaged frame for the material was then fed into the MATLAB program
with the corresponding average frame for the Sylgard as the reference. The result
obtained was an average value for the platelet intensity for the test material at a given
time and given flow condition. This averaging of the frames helped to decrease the errors
related to AC power source as well as the inability to differentiate between platelet
aggregates and WBCs. Moreover as the study was a comparative assessment for the
different polymers, the same errors were encountered in all the experiments and hence, it
was believed that the errors had a minimal effect on the conclusions.

The comparison between the various polymers and the control i.e. Sylgard was
carried out at steady and pulsatile shear rates and at three different time intervals of 5±1
min, 15±1 min, and 25±1 min at the same shear rate. The results for platelet deposition
on various polymer surfaces at different shear rates showed that there was a greater
platelet deposition at high shear rates (Figure 4.6 and Figure 4.8). Experiments carried
out on polyethylene (PE), polytetrafluoroethylene (PTFE), and silicone rubber (SR),
have shown that the platelet adhesion increases with increase in the shear rate
It was observed that the platelet intensity results at high pulsatile and high steady shear rates were higher at all times when compared with low and medium pulsatile and steady shear rates. It was also found that for all of the flow rates there was an increase in the platelet intensity with increase in the residence time. Each of the shear rate showed that the platelet intensity increased from 5±1 min interval to 25±1 min time interval. Experiments performed on polymer surfaces show that the platelet deposition increases with increase in the residence time (Balasubramanian 2002). One of the flow chambers was allowed to run for one hour and it was observed that the platelet intensity on the surface did not increase significantly, showing that the platelet deposition reached a maximum or a plateau after a certain amount of time. When the results for pulsatile and steady shear rates were compared, it was found that the results for both were same and had no significant differences at a given time interval. It was studied by Blusestein etal. (2000) that pulsatility in the flow increases the phenomenon of platelet attachment to the surface; however the platelet adhesion results did not show any significant increase in platelet intensity for pulsatile flow rates.

One-way ANOVA and post hoc tests carried out for various phospholipid modified Quatromers showed that the platelet adhesion results for PC, DMPC, and DDPC modified Quatromer are statistically insignificant. DMPC and DDPC are the saturated phospholipids and they have a compact bilayer as no kinks are present in their hydrophobic tail and this helps them to provide better hydrophilic properties to the surface. The saturation of their hydrocarbon tails decreases the water penetration and also prevents them from suffering from oxidation reactions or double bond cleavage. However, DDPC still may not be a good option for modification as the dissolving of
DDPC with Quatromer solution took a long time and lot of stirring. The third phospholipid, which showed excellent platelet adhesion, was PC which inspite having a double bond in its structure showed good hemcompatibility properties. The reason for this is that PC has a larger hydrophilic head when compared to the other phospholipids and the presence of this larger phospholipid head improved its surface modifying properties. Another phospholipid, which showed good platelet adhesion results, was DOPC, however this phospholipid has two double bonds in its tail structure and this may result in increased oxidation to form free radicals or peroxides. EPC has shown to have higher oxidation resistance due to the presence of ethanolamine in its structure however; the platelet adhesion results were not that good. The last phospholipid i.e. DAPC has eight double bonds in its structure and it is highly susceptible to cleavage of the double bonds forming unstable peroxides in vivo. Also, DAPC did not showed good platelet adhesion results and hence, it was ruled out.

One of the most important issues in deciding which phospholipid to consider for modification of Quatromer was the cost of the phospholipids. Phospholipids are quite expensive and there use may result in increased production cost of the biomedical implant. Table 5.1 shows the prices of the six different phospholipids used in the modification process of Quatromer. It can be observed that the price of DMPC is one-third when compared to the other phospholipids in consideration. Hence looking at the different advantages of DMPC such as low platelet adhesion, high oxidation resistance, stability of CH bonds, and low cost, it looks to be the most promising phospholipid for Quatromer modification when compared to the others.
Table 5.1 Prices for the various phospholipids used in modification of Quatromer

<table>
<thead>
<tr>
<th>Material</th>
<th>Price ($)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphatidylcholine (PC)</td>
<td>$26.00/25 mg</td>
</tr>
<tr>
<td>Ethanolaminephosphatidylcholine (EPC)</td>
<td>$37.86/25 mg</td>
</tr>
<tr>
<td>1,2-Dimyristoyl-rac-glycero-3-phosphocholine (DMPC)</td>
<td>$9.20/25 mg</td>
</tr>
<tr>
<td>1,2-Didodecanoyl-rac-glycero-3-phosphocholine (DDPC)</td>
<td>$42.30/25 mg</td>
</tr>
<tr>
<td>1,2-Dilinoleoyl-rac-glycero-3-phosphocholine (DOPC)</td>
<td>$23.00/25 mg</td>
</tr>
<tr>
<td>1,2-Diarachidonoyl-rac-glycero-3-phosphocholine (DAPC)</td>
<td>$101.00/25 mg</td>
</tr>
</tbody>
</table>

On comparing the surface modification technique and phase mixing technique to modify the Quatromer with phospholipid, the phase mixing technique would be the choice due to its numerous advantages in imparting long-term hemocompatibility. The various advantages such as homogenous or uniform distribution of phospholipid throughout the Quatromer material, formation of bilayers, and passive migration of inside phospholipid bilayers towards the surface on aging of the surface phospholipids, shows 3% phospholipid/Quatromer phase mixing technique to be a better choice for Quatromer modification.
6.0 Conclusion

The use of present prosthetic heart valves has been associated with incidence of structure failure, calcification, thrombosis, thromboembolism, and anticoagulant complications. This requires the need to look for a new class of hybrid biomaterial, which would have a combination of excellent material strength and durability as well as superior hemocompatibility. It has been observed that the present mechanical valves, possess the required material durability, however they fail when the surfaces comes in contact with blood leading to thrombosis and anticoagulant complications. The other type, bioprosthetic valves, has near native blood flow characteristics, yet they suffer from material degradation due to the deposition of calcium on the leaflets. Thrombosis and structural failure are clearly problems associated with the choice of material. The polymer heart valves with a superior biomaterial have the potential to eliminate the complications encountered in mechanical and bioprosthetic heart valves. A polymer composite having high resistance to oxidation reactions and excellent blood compatibility properties may help in the development of the next generation artificial heart valves. The Quatromer modified with phospholipids has the potential to be a superior biomaterial for artificial implants. The phospholipid modification would help to improve the blood compatibility properties as well as the material properties of the Quatromer.

In this study the potential of a new Quatromer phospholipid composite was evaluated to improve the long-term blood compatibility of a trileaflet heart valve material. This composite was prepared by mixing 3% phospholipid by weight with
Quatromer and the hemodynamic and mechanical properties of the composite were evaluated. Six different saturated and unsaturated phospholipids with varying hydrocarbon chain lengths were used to modify the Quatromer. The primary findings of the study show that phospholipid modification tends to increase the wettability of the highly hydrophobic Quatromer surface. The surface analysis results show that phospholipid modification of Quatromer surface decreases the contact angle significantly and also leads to an increase in the carbonyl groups on the surface, which correlates, with increased endothelial growth on the surface. The improved surface properties of phospholipid modified Quatromer are further supported by comparative platelet adhesion tests performed on Quatromer and phospholipid modified Quatromer. The platelet adhesion tests carried out using a parallel flow chamber showed that the phospholipids are capable of reducing the platelet adhesion on the surface by ten times. The mechanical tests performed on the phospholipids showed that the addition of phospholipids tend to decrease the modulus of elasticity of the Quatromer, however in the polymer leaflets, 95% of the load is carried by the mesh and hence, a decrease in the elasticity of Quatromer on phospholipid addition should not be a problem. The platelet addition tests showed that the DMPC proved to be the best phospholipid as it had higher oxidation resistance, significantly low platelet adhesion, high wettability, and ordered configuration. The phospholipid modification also helped in imparting secondary benefits to the Quatromer material, such as reduced tackiness or surface friction, smooth and homogeneous surface. The advantage of achieving reduced Quatromer tackiness is important in efficient manufacturing of the leaflets.
Based on the above findings, it can be concluded that the modification of Quatromer with DMPC would provide promising results in improving the long-term hemocompatibility of the polymer and increase its potential to be used in making polymeric heart valves. Studies still need to be carried out to assess an optimum concentration of phospholipid in the Quatromer material. Also, it is recommend that a more efficient way of fabricating DMPC modified leaflets may be devised, which would prevent any denaturizing or reorientation of the functional groups on the surface. One of the problems with fabrication of DMPC modified leaflets using compression molding would be operation at high temperatures. It has been discussed earlier that high temperatures tend to increase the disorderness of the bilayers in the phospholipid and hence, an alternate fabrication technique should be developed to prevent change of phospholipid properties at high temperatures. Peeling tests for DMPC modified Quatromer should be performed to determine adhesion force at the point of contact between the stent and the leaflets. Another important step would be to carry out in vivo pre-clinical testing of synthetic heart valves fabricated using this composite material. The platelet adhesion tests for the Quatromer as well as the DMPC modified Quatromer has been performed using in vitro controlled study with flat rectangular specimens, however, in vivo studies may show different results for both as the study would have the specimens in a leaflet shape (having curvatures). Hence, preclinical in vivo tests are required to better evaluate DMPC modified Quatromer’s potential in reducing platelet adhesion on the surface.

Basically, it has been observed that the reduced thrombotic potential of the Quatromer is due to the presence of phospholipids in the bulk material; however these
phospholipids are susceptible to oxidation reactions over time. One of the possible ways to overcome this shortcoming is to form a hemocompatible amphiphilic block polymer where a highly hydrophobic polymer is linked with a highly hydrophilic polymer. Thus, an amphiphilic block polymer i.e. poly (2-methacryloyloxyethyl phosphorylcholine (MPC)-block-styrene) can be fabricated, which would have the hydrophobic styrene linked with the hydrophilic MPC polymer. This kind of amphiphilic polymer may possess good blood compatibility properties, which may last over a long period of time. Another, possible way to enhance the Quatromer material properties would be attaching a hydrophilic PEG (polyethylene glycol)/phospholipid mixture, which may help to render more oxidation resistance as well as a hemocompatible surface. Hence, with the optimum material properties and reliable fabrication process, a Quatromer heart valve with a potential to mimic natural heart valve function and structure can be obtained.

American Heart Association’s 2003 Heart and Stroke Statistical Update.


Appendices
APPENDIX A – Protocols for specimen preparation
1. **Preparation of 20% Quatromer in toluene solution**
   a. Dry Quatromer pellets in oven at 70ºC for 24 hours.
   b. Take a 250 mL glass jar with black top and Teflon lid liner with a magnetic stirrer in it.
   c. Add to the glass bottle:
      i. Quatromer 40 g
      ii. Toluene 160 g
      iii. Mix with or without heat (70ºC) until dissolved.
      iv. Remove from mixer and replace lid.
   d. Label bottle with 20% Quatromer in toluene and date mixed.

2. **Preparation of Dogbone Specimens**
   a. Clean the stainless steel base plate, stainless steel rectangular blocks, and brass gaskets with alcohol pads.
   b. Dry the components of the plate assembly with Kimwipes.
   c. Put the brass gaskets on the base plate and the stainless steel rectangular blocks on the brass gaskets, and screw them together.
   d. In the hood, wearing a respirator and latex gloves, fill a graduated pipette with 20% Quatromer solution in toluene prepared using the above method.
   e. Pour 5.0mL of the 20% Quatromer solution in each of the rectangular cavity to achieve a specimen thickness of approximately 0.3mm.
   f. Prepare sure that the liquid in the cavity has no bubbles in it.
   g. Place the plate assembly horizontally in the oven at 70ºC, and it to dry for 4 hours.
   h. On removal from the oven, allow the dish to cool to room temperature
   i. Cut rectangular samples from each of the cavity using a blade.
   j. Place the specimen on the dogbone-cutter, and punch the dogbone shape out using a hammer.
   k. Apply Johnson’s baby powder to the Dogbone specimens, and number the specimens.
l. Place them in a ziplock bag and label them 20% Quatromer with date of manufacture, specimen numbers contained, and volume of solution used to prepare the specimen.

3. **Preparation of 3% phospholipid/Quatromer composite**
   a. Take a 10 mL glass jar with black top and Teflon lid liner with a magnetic stirrer in it.
   b. To prepare 3% phospholipid/Quatromer composite, add to the glass bottle:
      i. 20% Quatromer in Toluene (1 g Quatromer and 4 g Toluene) 5 g Phospholipid 30 mg
      ii. Mix until dissolved.
      iii. Remove from mixer and replace lid.
   c. Label bottle with 3% phospholipid/Quatromer mixture and date mixed.

4. **Preparation of phospholipid surface modified Quatromer:**
   a. Take a 50 mL glass jar with black top and Teflon lid liner with a magnetic stirrer in it.
   b. To prepare 20% phospholipid/Quatromer, add to the glass bottle:
      i. 20% Quatromer in Toluene (5 g Quatromer and 20 g Toluene) 25 g
      ii. Phospholipid 1 g
      iii. Mix until dissolved.
      iv. Remove from mixer and replace lid.
   c. Label bottle with 20% phospholipid/Quatromer mixture and date mixed.
   d. Take another 50 mL glass jar with a magnetic stirrer.
   e. Dip rectangular 20% Quatromer strips prepared using plate assembly in the 20% phospholipid/Quatromer mixture at 50°C for 1 hour.
APPENDIX B – Protocols for Mixing Chemical Solutions
**Heparin**

Ingredients:
- 50 mg of heparin sodium
- 166.5 mL of 0.9% NaCl

Mix together and pass through a 22-μm membrane filter. Solution should be stored at 4°C. Using 5 mL of Heparin solution corresponds to 270 units of Heparin

**Mepacrine**

Ingredients:
- 4.7 mg of Mepacrine
- 1 mL of Water

Mix 4.7 mg of Mepacrine in 1 mL of water to prepare a stock solution of 10 mM Mepacrine in water and store it at 4°C in a foil covered tube.
APPENDIX C – Protocol for Mepacrine Labeling
**Syringe Preparation for Blood Withdrawal**

1. Label three 60 ml syringes as Syringe 1 – Heparin, Syringe 2 – Heparin, and Syringe 3 – Heparin.
2. Extract 5 ml of Heparin solution (270 units) into Syringe 2-Heparin, Syringe 2–Heparin, and Syringe 3-Heparin.
3. Give all three syringes to the phlebotomist for blood withdrawal.
4. Collect 50 ml of blood into all the three syringes filling to a total of 55 ml.
5. Place syringes on rocker
6. Transfer the blood from the three syringes into a 500 mL plastic bottle.
7. Cover the bottle with Aluminum foil.
8. Take the 10mM Mepacrine solution and add 150μL of Mepacrine to 150 mL of blood.
10. Invert the bottle gently a couple of times to mix the platelets with Mepacrine.
APPENDIX D – AutoCAD Drawings for Blood Flow and Damping Chambers
Dr. By: Tejas Choksi

05 February 2004

Material: Lexan

Note: All dimensions in millimeters unless otherwise stated.
Florida International University
Biomedical Engineering Institute

Title: Flow Chamber-Top Plate
Dr. By: Tejas Choksi
05 February 2004
Scale: 1:1
Material: Lexan

Note: All dimensions in millimeters unless otherwise stated
Note: All dimensions in millimeters unless otherwise stated.
APPENDIX E – Institutional Review Board (IRB) for Research Involving Human Participants
IRB RESEARCH PROPOSAL

1. Research Objectives

Heart valve prostheses have been used successfully since 1960; however, the two classes of prostheses that are commercially available today (mechanical and bioprosthetic) have their associated shortcomings. The research outlined in this proposal aims to lead to the development of a third class of valves, polymer trileaflet valves, that combine the advantages of the mechanical and bioprosthetic valves for a better alternative for heart valve replacement. The overall project aims to determine an appropriate material, an appropriate design, and a repeatable and reliable manufacturing process for the valve. The polymer chosen for the development of this valve (SIBS) has established biostability, but the thrombogenic potential of the material is unknown. It is the intention of this particular research study to compare the thrombogenic potential of SIBS, polyurethane, and porcine or pericardial valve material by measuring platelet deposition under controlled in vitro flow conditions. Blood samples obtained from human volunteers will be pumped through chambers containing test materials, and the deposition of platelets on the materials will be measured and compared.

2. Subject Recruitment

Normal, healthy, non-smoking subjects who are over 18 and have not consumed alcohol, aspirin, or vitamin E within the last 24 hrs, and who are not pregnant will be recruited from the local student population. Approximately twenty subjects will be recruited.

3. Benefits

(a) No benefits to the subject.
(b) Importance of knowledge to be gained.
The results of this research will provide data regarding the feasibility of the new SIBS material as a potential material for use in artificial heart valves. If this research is successful, artificial heart valve recipients will live longer and maintain a higher quality of life by the elimination of repeat surgeries or the use of anticoagulant therapy.

4. Risks to Subjects

The study requires the collection of blood samples from volunteers. For each experiment, approximately 150 mliters of blood will be collected from the subject by venipuncture using sterile equipment. Venipuncture presents minimal risk of infection, discomfort, and possible bruising. Care will be taken to ensure that an individual subject will not be sampled more than twice in one week and in an amount not exceeding 450 mliters in an eight-week period. The procedure will be performed at FIU’s Cardiovascular Engineering Center by a licensed phlebotomist. A screening procedure, by means of a survey, will determine the health of the patient in terms of anemia, HIV, and Hepatitis B status. The patient will be provided a place to lie comfortably during the venipuncture procedure, and juice and cookies will be provided following the procedure.

All staff in FIU’s Cardiovascular Engineering Center that will be handling the blood will be vaccinated against Hepatitis B, and they will be required to wear latex gloves and lab coats at all times. The rooms are well labeled to caution against biohazards.

5. Informed Consent

Written informed consent will be obtained from each subject before each collection of material. (see enclosed informed consent form).

6. Confidentiality of Data
Data obtained from the experiments will be reported as averages and will not disclose individual information. The data obtained will include platelet counts, red blood cell counts, and platelet deposition counts. In addition, the data will be coded and the key to the code will be kept in a locked cabinet with access only available to the PI. All health surveys and informed consent documents will also be kept in the locked cabinet; however, none of the data obtained is expected to be detrimental to the subject.

7. Methods and Procedures:

Following a screening procedure to determine overall health of a subject, approximately 150 mliters of blood will be collected from each subject by venipuncture using sterile equipment. This sample of blood will then be used in the experiments described in #1 above. Care will be taken to ensure that an individual subject will not be sampled more than twice in one week and in an amount not exceeding 450 milliliters in an eight-week period. The procedure will be performed at FIU’s Cardiovascular Engineering Center by a licensed phlebotomist.

8. Stimulus Materials

None.
CONSENT TO PARTICIPATE IN A RESEARCH STUDY

Title: a Novel Polymer Trileaflet Heart Valve

You are being asked to be in a research study to be conducted at Florida International University during the 2003-2004 academic year. The investigator of this study is Tejas Choksi and she is a student at Florida International University (FIU). The study will include 20 subjects who are students studying at FIU. Your participation would require 10 minutes of your time. We are looking to gain further understanding of the thrombogenic potential of SIBS (polymer used for trileaflet heart valve prosthesis) material modified with various surfactants by measuring platelet deposition under controlled in vitro flow conditions.

If you decide to be a part of the study we will tell you what day and time to come to the Cardiovascular Engineering (CVEC) lab. The procedures for drawing blood will be as follows: 150 milliters of blood will be collected by venipuncture from my forearm, by a licensed phlebotomist, into three 50 cc syringes containing heparin as an anticoagulant. This blood sample will then be used in blood flow experiments designed to measure platelet deposition. The venipuncture procedure presents a minimal risk of infection, discomfort and possible bruising. In case you get hurt or upset a licensed phlebotomist would be there to provide you with the necessary help needed.

There is no cost or payment to you as a subject. You will not get any direct benefit from being in the study. However, your help will give us information about the thrombogenic potential of SIBS polymer, which would serve as a potential material in the development of a novel trileaflet heart valve used to cardiovascular disorders. You will get juice and cookies as a token for being in the study.

Your data regarding the blood sample (platelet count, red blood cell count, and platelet deposition count) will be kept confidential. The data will be coded and the key to the code will be kept in a locked cabinet with access only available to the PI. You may ask questions about the study at any time and any questions will be answered to your satisfaction. You may choose not to participate in this research project at any time with no negative consequences. You may also choose to stop your participation before the procedure finishes. Even if you do not finish the procedure you will get the juice and cookies.

If you would like more information about this research after you are done, you can contact Dr. Richard T. Schoephoerster, Principal Investigator of this research or me at 305-348-3722. If you would like to talk with someone regarding the rights of research subjects, you should contact Dr. Bernard Gerstman, Chairperson of the FIU Institutional Review Board at 305-348-3115 or 305-348-2494

Your signature below indicates that all questions have been answered to your liking. Your are aware of your rights and you would like to be in the study.

Signature of Participant ___________________________ Printed Name ___________________________ Date __________

I have explained the research procedure, subject rights, and answered questions asked by the participant. I have offered him/her a copy of this informed consent form.

Signature of Witness ___________________________ Printed Name ___________________________ Date __________
APPENDIX F – Matlab Program
function bkgndcorrect(ref, actdata, fileout, strp)
% This function is used to background correct TIF images
% from the stent project
% 1st input - reference inlet TIF file
% 2nd input - actual data TIF file
% 3rd input - output file name for storage
% 4th input - number of strips of each frame
% For 4th input - enter 1 if data is to be regressed on a line by line basis
% For 4th input - enter 2 if data is to be regressed on the average from 252 lines (504/2)
% Input reference file read
A=imread(ref,'tif');
A=double(A);
[M,N]=size(A);
n=1:1:N;
m=floor(M/strp);
% Average of intensity from input reference file
tot=sum(sum(A));
avg=tot/(M*N);
% Actual data file read
B=imread(actdata,'tif');
B=double(B);
% Average data of the required number of rows (based on number of strips as input)
dataInp=zeros(M,N);
dataOut=zeros(M,N);
if strp==1
  dataInp=A;
dataOut=B;
else
  ind=1;
  for i=1:m:M
    j=i
    while (j<=(i+m-1) & (i+m-1)<=M)
      dataInp(ind,:)=dataInp(ind,:)+A(j,:);
dataOut(ind,:)=dataOut(ind,:)+B(j,:);
j=j+1;
    end
  end
  dataInp(ind,:)=dataInp(ind,:)/m;
dataOut(ind,:)=dataOut(ind,:)/m;
  ind=ind+1;
end
[F,G]=size(dataInp);
% Regressing 4th order polynomial to data from input reference file
% across all rows
for i=1:F
  ...
end
dataO=mean(dataInp);
coeff(i,:)=polyval(polyfit(n,DATAO,4),n);
end
% Background correction: (data/ref)*avg
for i=1:F
  for j=1:G
    C(i,j)=dataOut(i,j)*avg/coeff(i,j);
  end
end
Cavg=mean(C);
Cavg=Cavg';
% Write background corrected data into output file
fid=fopen(fileout,'w');
for i=1:size(Cavg)
  count=fprintf(fid,'%8.5fn',Cavg(i));
end
fclose(fid);
APPENDIX G – Statistics
Table F1. One-way ANOVA test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 5±1 min time interval

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>21601.947</td>
<td>39</td>
<td>553.896</td>
<td>600.714</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>331.943</td>
<td>360</td>
<td>.922</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>21933.890</td>
<td>399</td>
<td>.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table F2. One-way ANOVA test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 15±1 min time interval

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>52881.141</td>
<td>39</td>
<td>1355.927</td>
<td>837.972</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>582.518</td>
<td>360</td>
<td>1.618</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53463.659</td>
<td>399</td>
<td>.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table F3. One-way ANOVA test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 25±1 min time interval

<table>
<thead>
<tr>
<th>ANOVA</th>
<th>Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between Groups</td>
<td>107145.4</td>
<td>39</td>
<td>2747.317</td>
<td>1622.257</td>
<td>.000</td>
</tr>
<tr>
<td>Within Groups</td>
<td>609.666</td>
<td>360</td>
<td>1.694</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>107755.0</td>
<td>399</td>
<td>.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table F4. Tukey post hoc test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 5±1 min time interval

| MATERIAL                  | N | 1   | 2   | 3   | 4   | 5   | 6   | 7   | 8   | 9   | 10  | 11  | 12  | 13  | 14  | 15  | 16  | 17  |
|---------------------------|---|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| DDPC+Q-LS                 | 10| 2.49|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DMPC+Q-HP                 | 10| 2.76| 2.76|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DDPC+Q-HP                 | 10| 2.88| 2.88|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DMPC+Q-HS                 | 10| 2.84| 2.84|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DOPC+Q-HS                 | 10| 3.12| 3.12| 3.12|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DMPC+Q-MS                 | 10| 3.13| 3.13| 3.13|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| PC+Q-LS                   | 10| 3.16| 3.16| 3.16|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DDPC+Q-LS                 | 10| 3.15| 3.15| 3.15|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DOPC+Q-MS                 | 10| 3.18| 3.18| 3.18|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DMPC+Q-LS                 | 10| 3.46| 3.46| 3.46| 3.46|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| PC+Q-MS                   | 10| 4.18| 4.18| 4.18| 4.18| 4.18|     |     |     |     |     |     |     |     |     |     |     |     |     |
| DDPC+Q-LS                 | 10| 4.23| 4.23| 4.23| 4.23|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DMPC+Q-MS                 | 10| 4.34| 4.34| 4.34| 4.34| 4.34|     |     |     |     |     |     |     |     |     |     |     |     |     |
| PC+Q-MS                   | 10| 4.77| 4.77| 4.77| 4.77|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DOPC+Q-MS                 | 10| 4.92| 4.92| 4.92|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DOPC+Q-LS                 | 10| 5.12| 5.12| 5.12|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DOPC+Q-MS                 | 10| 5.14| 5.14| 5.14|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| PC+Q-HP                   | 10| 5.52| 5.52| 5.52|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DAPC+Q-LS                 | 10| 5.68| 5.68| 5.68| 5.68|     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DAPC+Q-MS                 | 10| 6.97| 6.97| 6.97|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-LS                  | 10| 7.24| 7.24| 7.24|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DAPC+Q-MS                 | 10| 7.30| 7.30|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| DAPC+Q-LS                 | 10| 7.58| 7.58|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-LS                  | 10| 7.78| 7.78|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-LS                  | 10| 8.28| 8.28|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-MS                  | 10| 8.52| 8.52| 8.52|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-MS                  | 10| 8.59| 8.59| 8.59|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-LS                  | 10| 8.67| 8.67| 8.67|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-MS                  | 10| 9.27| 9.27|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| EPC+Q-LS                  | 10| 10.24|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-MP                      | 10| 12.21|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-MS                      | 10| 13.39|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-LS                      | 10| 15.44|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-HS                      | 10|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-LS                      | 10| 16.42|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-MP                      | 10| 19.71|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-HS                      | 10| 21.92|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-LS                      | 10| 22.14|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-MP                      | 10| 23.43|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| S-MS                      | 10| 27.05|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-MS                      | 10|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |
| Q-HS                      | 10| 30.43|     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |     |

Means for groups in homogeneous subsets are displayed.
a. Uses Harmonic Mean Sample Size = 10.000.
Table F5. Tukey post hoc test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 15±1 min time interval.
Table F6. Tukey post hoc test for Sylgard (control), Quatromer, and different phospholipid modified Quatromers at different shear rates for 25±1 min time interval.

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Means for groups in homogeneous subsets are displayed.

a. Uses Harmonic Mean Sample Size = 10.00.