DESIGN AND OPTIMIZATION OF A BLOOD VESSEL MIMIC BIOREACTOR
SYSTEM FOR THE EVALUATION OF INTRAVASCULAR DEVICES
IN SIMPLE AND COMPLEX VESSEL GEOMETRIES

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by

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ABSTRACT

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by

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Coronary artery disease affects millions of people and the ability to detect and treat the disease is advancing at a rapid rate. As a result, the development of intravascular technologies is the focus of many medical device manufacturers. Specifically, coronary stent implantation is being performed in an increasing number of patients and a number of new stent designs have been introduced to the market, resulting in the need for improved preclinical testing methods. An *in vitro* tissue engineered “blood vessel mimic” (BVM) system has previously been established and its feasibility for the initial testing of newly emerging intravascular technology has been demonstrated. There are limitations that exist with this original design, however, and the focus of this thesis was to both improve and expand upon the original model. Therefore, research was conducted based on two specific aims. The first aim was to develop a more ideal BVM system to accommodate a wider range of stent lengths and diameters, while allowing for easy graft insertion and seal-ability. The second aim was to develop next generation BVM systems,

focused on future needs and technology, such as long, angulated and bifurcated geometries.

The work described in this thesis demonstrates that a BVM chamber can be created which has the advantages of easy graft insertion and seal-ability, as well as the ability to accommodate varying sizes of vessel scaffolds, all while maintaining the needs of a tissue engineering bioreactor system. The next generation BVM systems presented demonstrate that the BVM concept can be expanded to meet the needs of long, angulated and bifurcated geometries. Overall, the work in this thesis describes the design and optimization of an *in vitro* blood vessel mimic bioreactor system for the evaluation of intravascular devices, specifically coronary stents, in simple and complex vessel geometries.

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DEDICATION

For my Dad,

Jeffrey D. Leifer

February 29, 1952- July 15, 2001

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1. INTRODUCTION

Overview

Coronary stent implantation is being performed in a large number of patients with a wide spectrum of clinical and lesion characteristics. From single and multiple vessel disease to bifurcated, angulated and long lesions, the variation and complexity of coronary artery disease and the advanced capabilities to detect and determine the extent and severity has led to an increased interest in intravascular device innovation (Zhu, et al. 2008; Kini 2006). A variety of stent designs currently exist and continuous efforts are being made to improve both stent design and placement procedures (Kandzari, Tchong and Zidar 2002; Kraitzer, Kloog and Zilberman 2008). Smaller delivery catheters and advanced delivery systems are also being introduced and have allowed the treatment of coronary artery disease and stent placement to become less invasive and easier on both the patient and physician (Yamasaki, et al. 2008; Takeshita, et al. 2008).

With the transition from open heart surgery to balloon angioplasty and stent placement, minimally invasive procedures have become the most often used technique for treating many cardiovascular diseases (American Heart Association 2008), which in turn has lead to a high demand for preclinical assessments of new intravascular devices (Kandzari, Tchong and Zidar 2002; Cardinal, et al. 2006). The explosive growth of coronary stenting has fueled a surge in the number and types of coronary stents and delivery systems being designed and introduced to the market. Specifically in the area of coronary stents, a wide range of stent materials, coatings, drugs, and configurations currently exist, with future developments accelerating at a rapid pace (Kraitzer, Kloog

and Zilberman 2008). Some of the most exciting advances can be seen in new stents designed specifically to treat bifurcation lesions, an area that has been very challenging for physicians, and new delivery systems which can deploy long or multiple stents at one time in order to better treat patients with long lesions or lesions in multiple vessels (Ikeno, Buchbinder and Yeung 2007; Stella, et al. 2008). This surge in stent technology has resulted in the need for new and advanced preclinical testing methods that provide medical device manufacturers and researchers with the ability to quickly evaluate tissue response to new and novel stent designs and stenting techniques.

Coronary stents are constantly evolving devices that possess great potential, but each innovation and each new design that is developed must be fully evaluated before clinical applications are possible. Through the creation of an *in vitro* tissue engineered blood vessel construct, previously developed and called a “blood vessel mimic” (BVM), a model system can be established for the initial testing of newly emerging intravascular technology (Cardinal, et al. 2006). The goal of this thesis is to not only expand upon the original BVM model, but to develop new and innovative models for the evaluation of devices in more complex geometries such as bifurcations, long and angulated vessels.

The following introduction will provide the background and foundation on which this thesis is based. A review of coronary artery disease will be given followed by current diagnosis and treatment procedures. Coronary stents will be discussed in detailed, outlining the materials utilized, shapes, configurations, coatings and drugs used in their design. Other intravascular devices, delivery systems and technologies will also be addressed along with an overview of intravascular device preclinical testing methods and the BVM system specifically. The introduction will conclude with a summary of the

specific aims for this thesis, which will lead into the individual chapters and the models, experiments and studies that were built and performed.

Coronary Artery Disease

Pathology

In order to understand why intravascular technologies, specifically stents, have become the treatment of choice for many cardiovascular diseases, and why the medical community has focused so much attention on creating and testing intravascular devices to treat these diseases, one must appreciate the history and prevalence of the disease itself. Coronary artery disease (CAD), also known as coronary heart disease (CHD), is the number one killer of both men and women in the United States, affecting an estimated 16 million people (American Heart Association 2008). In 2008 alone, the direct and indirect cost of treating CAD is estimated at \$156.4 billion (American Heart Association 2008), making it the primary focus of many medical device manufacturers.

The coronary arteries are small blood vessels that run along the surface of the heart and carry blood which supplies oxygen and nutrients to the heart muscle. The anatomical characteristics of coronary arteries vary considerably, ranging from straight simple geometries to extremely angulated bends and curves as well as bifurcations and long lesions (Ellis, et al. 1990; Zhu, et al. 2008; Smith, et al. 2001). Depending on the individual, the anatomical structure of the coronary arteries can include all or some of the characteristics identified above.

Coronary artery disease can be described as atherosclerosis of the coronary arteries (Falk 2006; Noll 1998). Atherosclerosis occurs when the coronary arteries become narrowed or blocked by fatty deposits called plaque, restricting blood flow to the heart (Deconinck, et al. 2008). A healthy artery and an artery affected by atherosclerosis

are illustrated below in Figure 1.1. Without adequate blood, the heart becomes starved of oxygen and vital nutrients it needs to work properly. If left untreated coronary artery disease leads to chest pain, heart attack, heart failure and ultimately death (American Heart Association 2008). Therefore, action must be taken to reopen the narrowed vessels and restore blood flow.

Figure 1.1- Atherosclerosis of the coronary arteries. Plaque accumulation on the artery wall results in restricted blood flow to the heart.

(Permission granted to reprint image from the National Heart, Lung, and Blood Institute as a part of the National Institutes of Health and the U.S. Department of Health and Human Services; see Appendix G)

For many years, the process of atherosclerosis has been viewed simply as the accumulation of lipids within the artery wall, however it is now understood that atherosclerosis is a complex inflammatory disease (Libby, Ridker and Maseri 2002; Ross 1999; Noll 1998) involving endothelial cells (EC), smooth muscle cells (SMC), T-lymphocytes, B-lymphocytes, monocytes, leukocytes and macrophages (Stoll and Bendszus 2006; Falk 2006). This new insight into the role of inflammation in the development of atherosclerosis has initiated important new areas of research in the treatment of coronary artery disease, most notably in the area of pharmaceuticals (Deconinck, et al. 2008). As will be discussed in detail, the use of pharmaceuticals on the surface of stents in an attempt to prevent or slow the progression of atherosclerosis is a recent technology and an area that is still undergoing research and in need of preclinical testing options.

Diagnosis

Coronary artery disease develops slowly, often without any signs or symptoms prior to a heart attack. However, there are a number of symptoms that can help diagnosis CAD and lead to proper treatment (American Heart Association 2008). The most common symptom of coronary artery disease is chest pain, also known as angina pectoris, or simply angina (Khan 2005; Crawford 2002). Angina results from the heart not getting enough blood and oxygen and is described as chest discomfort, heaviness, tightness, pressure, aching, burning, numbness, fullness, or squeezing. It is most often felt in the chest, but may also be felt in the shoulders, arms, neck, back or jaw. Shortness of

breath is a second symptom and is most often experienced during physical activity. Due to long-term lack of blood and oxygen, the heart becomes weak and is unable to pump blood throughout the body. Other less common symptoms of CAD include heart palpitations, irregular or rapid heartbeat, weakness, dizziness, nausea, and sweating. Whatever the symptom, diagnosis is the first step to treatment.

Diagnosis of coronary artery disease begins with a complete review of medical and family history and a thorough physical examination (Crawford 2002). Although both these methods can predict the likelihood of an individual's chance of having the disease a diagnostic test must be performed in order to verify the diagnosis. Often the first diagnostic tests chosen are an electrocardiogram (ECG or EKG) or echocardiogram (ECHO), since these tests are relatively low cost, simple, and non-invasive, providing fast information about the function of the heart. Stress tests, nuclear imaging, computerized tomography (CT) scans, positron emission tomography (PET) scans, magnetic resonance imaging (MRI) and other non-invasive diagnostic tests may also be performed, with varying degrees of imaging and informational capabilities. Currently, however, one of the most often used diagnostic tests and the reference against which all other diagnostic imaging tests are compared is the coronary angiogram, providing a direct image of the coronary arteries themselves (Budoff, et al. 2008; Sun and Jiang 2006). Coronary angiography is a minimally invasive imaging procedure in which a catheter is guided into the coronary arteries, a process often referred to as cardiac catheterization, dye is injected, and x-ray images are taken as the contrast material moves through the heart's chambers, valves and major vessels. The resulting images are used to identify sites of narrowing or blockage in the coronary arteries and provide a visual picture of the location

and severity of coronary artery disease. Coronary angiography is illustrated below in Figure 1.2.

Figure 1.2- Coronary angiography. A catheter is guided into the coronary arteries, dye is injected, and x-ray images are taken as the contrast material moves through the heart.

(Permission granted to reprint image from the National Heart, Lung, and Blood Institute as a part of the National Institutes of Health and the U.S. Department of Health and Human Services; see Appendix G)

Using angiography alone, however, allows only a limited quantitative analysis of the vessel lumen blockage from the silhouette image obtained; and although angiography reveals artery narrowing, it cannot differentiate between the vessel layers or identify the presence of plaque on the artery walls (Kern, et al. 2006; Wahle, et al. 2006). Therefore,

more advanced imaging techniques are often performed to supply additional information about the coronary arteries that angiography alone cannot provide.

Intravascular ultrasound (IVUS) is an imaging procedure that can be performed simultaneously with coronary angiography in order to obtain detailed images of the walls of the coronary arteries. During IVUS a miniature sound-probe is positioned on the tip of a coronary catheter. The catheter is threaded through the coronary arteries and using high-frequency sound waves detailed images of the inside walls of the arteries are created. These images show cross-sections of the lumen revealing the layered structure of the vascular wall and provide information about the composition of the lesions including plaque shape and size (Cardinal, et al. 2006; Schoenhagen and Nissen 2002).

No matter what technique is used, the goal of coronary imaging is to identify the location, characteristics, number and severity of lesions present in a patient diagnosed with coronary artery disease. Treatment decisions are based on these findings and specific treatment options are available for a number of lesion characteristics. Depending on the number, severity and location of the blockages found during the diagnostic procedure, the physician can recommend the appropriate treatment.

Treatment of Coronary Artery Disease

Treatment of coronary artery disease can include lifestyle changes, medications, and medical procedures. The goal of each treatment is to relieve symptoms and reverse or slow the progression of the disease. Depending on the severity of the disease, a combination of treatments may be chosen. Although the focus of this thesis is on interventional medical procedures and intravascular devices, it is important to realize that if CAD is caught early enough interventional procedures or surgery may not be needed which is the best scenario for both the patient and physician.

Lifestyle Changes and Medication

Simple lifestyle changes can often help prevent or control CAD and for some individuals these changes may be the only treatment needed. It is hard to determine which controllable risk factors are most important but, in general, high cholesterol, cigarette smoking, excess weight, sedentary lifestyle, high blood pressure, and diabetes seem to be the most significant (American Heart Association 2008; Crawford 2002). Therefore, diet changes, avoiding tobacco products, exercise, and weight loss can often reduce these risk factors and control the onset and development of CAD.

For some individuals, however, lifestyle changes may not be enough and medication may be prescribed. A number of medications are currently used, each with the goal of either treating the risk factors associated with CAD or treating the effects of the disease. Aspirin, a blood thinner, is recommended for almost everyone who has CAD, to

help reduce the risk of having a heart attack (Hayden, et al. 2002). Other medications include: anti-platelet agents and anticoagulants, which prevent the formation of blood clots and thin the blood; statins, which help lower cholesterol; nitrates and ACE inhibitors, which dilate the arteries and make it easier for blood to flow; beta-blockers and calcium channel blockers, which slow the heart rate and reduce blood pressure (Zoghbi, Dorfman and Iskandrian 2008). Unfortunately, when medications and lifestyle changes are not able to control symptoms or the narrowing progresses to a point that the heart muscle is at risk for damage, interventional procedures or surgery may be required.

Coronary Artery Bypass Grafting

A number of surgical procedures and interventional therapies have been developed over the past several decades to treat coronary artery disease, each with the goal of quickly and safely restoring blood flow to the heart. Coronary artery bypass graft surgery (CABG) was developed as a treatment for CAD in the late 1960's and is still a frequently used technique today (American Heart Association 2008). In this procedure a healthy vein or artery is removed from the patient's body and used to bypass the coronary blockage (Khan 2005; Mullany 2003). The saphenous vein, internal mammary artery and radial artery are used most often, however when no healthy segment can be found within the patient's body a synthetic graft material, such as expanded polytetrafluoroethylene (ePTFE), can also be utilized (Weyand, et al. 1999).

During CABG the patient's chest is surgically opened and the harvested artery or vein is connected between the aorta and a healthy area of the heart in order to provide a

pathway for the blood to flow around the site of the damaged and narrowed tissue. An illustration of CABG is shown below in Figure 1.3.

Figure 1.3- Coronary artery bypass grafting. A healthy vein or artery is connected between the aorta and a healthy area of the heart in order to bypass the coronary blockage and provide a pathway for blood to flow.

(Permission granted to reprint image from the American Heart Association/American Stroke Association; see Appendix G)

For many years, CABG had been considered the standard of care for treating CAD in patients at moderate to high risk of a heart attack (Hamm, et al. 1994). In many cases CABG is still the best choice of treatment. However, CABG can be a highly invasive procedure that is generally associated with long recovery times and hospital stays, as well as high cost (American Heart Association 2008; Mullany 2003). For many patients open heart surgeries, such as CABG, are not well tolerated and lead to

complications that are amplified by the heart's weakened condition. Thus less invasive methods to treat CAD have been developed.

Balloon Angioplasty

Percutaneous coronary intervention (PCI) is a minimally invasive procedure used to treat patients with diseased coronary arteries. The initial innovation was balloon angioplasty, originally referred to as percutaneous transluminal coronary angioplasty (PTCA). The first balloon angioplasty was performed in 1977 by the Swiss physician Andreas Gruentzig (King and Meier 2000; Gruentzig 1978) and the basic technique is still used today. During balloon angioplasty a physician inserts a flexible catheter with a balloon tip into the patient's vascular system, most often through the femoral artery at the groin, and maneuvers the catheter through the vascular system into the coronary arteries. At the site of the coronary lesion or blockage the balloon is inflated, compressing the plaque and stretching the artery wall, creating a wider channel and restoring blood flow to the heart. Balloon angioplasty is illustrated in Figure 1.4 below.

Figure 1.4- Balloon angioplasty. A flexible catheter with a balloon tip is maneuvered into the coronary arteries and inflated, compressing the plaque and stretching the artery wall restoring blood flow to the heart.

(Permission granted to reprint image from the National Heart, Lung, and Blood Institute as a part of the National Institutes of Health and the U.S. Department of Health and Human Services; see Appendix G)

Coronary angioplasty provides physicians with a simple, minimally invasive method of opening a blocked artery without having to open the patient's chest. This therapy has been widely adopted by physicians because it results in shorter hospital and recovery times compared to CABG and is well tolerated by most patients (American Heart Association 2008). However, while providing advantages over CABG, the long-term effectiveness of balloon angioplasty is limited by restenosis, or re-narrowing of the artery. Restenosis is observed to occur due to two primary causes: the elastic recoil of the artery wall, its shrinkage back to its original size, and neointimal proliferation, the formation of scar tissue (Kraitzer, Kloog and Zilberman 2008; Deconinck, et al. 2008). This statement is an oversimplification of the process of restenosis, and restenosis will be discussed in detail in the following paragraphs, but for now it is important to note these two key observations as they are part of the motivation for the following advancements in treatment.

Stenting

One of most significant innovations in percutaneous coronary intervention, and that which is still evolving today, was the introduction of the stent in the late 1980's (Waksman 2002; King and Meier 2000). Stents are tube shaped scaffolds, typically metal or polymer, which are used to physically hold the artery wall open and address the first primary cause of restenosis (Kraitzer, Kloog and Zilberman 2008; Kandzari, Tchong and Zidar 2002). In an attempt to prevent elastic recoil of the artery wall stents were incorporated into the balloon angioplasty procedure in the early 1990's. It is noteworthy

to mention, however, that the stenting of peripheral arteries had already been experimented with by Charles Dotter in the 1960's who hypothesized that the use of stents in coronary arteries was also possible (King and Meier 2000; Dotter 1969). It should also be noted that in this section only a basic definition and description of stent design is addressed. Coronary stents will be discussed in detail in subsequent sections.

During a percutaneous coronary interventional stenting procedure, a stent mounted on a balloon catheter is delivered to the lesion, or site of narrowing and blockage. The balloon is inflated expanding the stent, compressing the plaque and stretching the artery wall. The balloon catheter is then removed, leaving the stent behind to hold the vessel open, restoring blood flow to the heart. Stent placement is illustrated below in Figure 1.5.

Figure 1.5- Stent placement. A stent is mounted on a balloon catheter and maneuvered into the coronary arteries. The balloon is inflated compressing the plaque, expanding the stent and restoring blood flow to the heart.

(Permission granted to reprint image from the National Heart, Lung, and Blood Institute as a part of the National Institutes of Health and the U.S. Department of Health and Human Services; see Appendix G)

Bare Metal Stents

In 1986, Jacques Puel and Ulrich Sigwart inserted the first bare metal stent into a human coronary artery (King and Meier 2000; Sigwart, et al. 1987). Bare metal stents (BMS) are coil, mesh or slotted tubes consisting of interconnected struts that are inserted inside the narrowed artery and expanded to hold it open. Stents significantly lowered the occurrence of restenosis by preventing elastic recoil of the artery wall and quickly replaced the use of balloon angioplasty as the primary interventional therapy for CAD (Waksman 2002; King and Meier 2000).

In 1994 the first bare metal stents, Palmaz-Schatz and Gianturco-Roubin, were approved for use in the United States, and over the next decade, several generations of bare metal stents were developed. By early 2000, stenting was performed in over 80% of all PCI procedures reducing the restenosis rate to 15-20% (Holmes, et al. 2002; Waksman 2002; Kandzari, Tchong and Zidar 2002). Previously, restenosis rates following balloon angioplasty had been seen in 30-40% of patients (Silverman 2006; Waksman 2002) and often required re-intervention by physicians. Unfortunately, despite their widespread use, bare metal stents are plagued by the occurrence of in-stent restenosis, re-narrowing of the artery around the stent, with patients showing clinical evidence within the first 3-9 months after implantation (Holmes, et al. 2002). In-stent restenosis around an implanted stent is illustrated in Figure 1.6 below.

Figure 1.6- In-stent restenosis. Re-narrowing of the artery around the stent.

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Restenosis

Restenosis is defined as the arterial healing response after injury incurred during coronary angioplasty (Lowe, Oesterle and Khachigian 2002; Indolfi, et al. 2003). In-stent restenosis (ISR) is defined as the arterial healing response due to injury incurred during coronary angioplasty and the presence a stent. Both restenosis and in-stent restenosis are

the result of a complex series of biological events in response to the initial injury of the vessel caused by balloon expansion and the presence of a permanent stent implant (Lewis, Tolhurst and Stratford 2002).

The process of restenosis involves vessel elastic recoil, negative remodeling, thrombus, smooth muscle cell proliferation, and excessive extracellular matrix production (Weintraub 2007). Neointimal formation, also known as neointimal proliferation, is one of the main mechanisms of restenosis and composed mainly of proliferating smooth muscle cells and extracellular matrix formation (Lowe, Oesterle and Khachigian 2002). In addition, inflammatory responses, foreign body reactions, and thrombogenesis occur in the tissue surrounding the struts of the stent and contribute to in-stent restenosis (Karoussos, et al. 2002). In-stent restenosis is a major clinical problem associated with stenting. Therefore, many efforts are being made to reduce or eliminate its occurrence.

In recent years, drug eluting, coated and bioabsorbable stents have been introduced into interventional cardiology as a possible solution to the problem of in-stent restenosis. Researchers and medical device manufacturers are continuously testing a variety of drugs that are known to interrupt the biological processes that cause restenosis (Deconinck, et al. 2008; Kraitzer, Kloog and Zilberman 2008). Stent coatings can optimize the surface properties of metallic stents and serve as a vehicle for local drug delivery (Hara, et al. 2006). Biodegradable materials can be absorbed by the body, allowing a stent to act as a scaffold for a period of time and then slowly disappear once it is no longer needed (Mani, et al. 2007). As a result the evolution of the stent has moved away from being a purely mechanical object placed within the artery. With

pharmacologic and material advances, stents have become active devices that interact with their environment in complex ways.

Drug Eluting Stents

A recent innovation in percutaneous coronary intervention is the development of drug eluting stents (DES). Drug eluting stents address the second basic cause of restenosis by attempting to prevent or slow neointimal proliferation and the formation of scar tissue at the lesion site (Kraitzer, Kloog and Zilberman 2008; Waksman 2002; Lowe, Oesterle and Khachigian 2002). A drug eluting stent is a device that releases a single or multiple bioactive agents into the bloodstream that affect the tissue adjacent to the stent. The drugs used inhibit cell growth and proliferation and this advancement has resulted in improved patient outcomes due to reduced in-stent restenosis (Popma, et al. 2007). The specific drugs used to prevent restenosis and their incorporation into stent design will be discussed in detail in the following sections.

In 2001, drug eluting stents (DES) were introduced into percutaneous coronary interventional procedures as a strategy to minimize in-stent restenosis due to neointimal proliferation (Kraitzer, Kloog and Zilberman 2008; Deconinck, et al. 2008). In the data gathered so far drug eluting stents have been extremely successful in reducing in-stent restenosis rates from 15-20% to single digits (Holmes, et al. 2002; Waksman 2002; Kandzari, Tchong and Zidar 2002). At present, 90% of all stents placed in the United States are drug eluting (Popma, et al. 2007). One disadvantage, however, is the development of late in-stent restenosis and thrombosis, which occur after the drug has

been completely eluted and is no longer present in the coronary stent (Deconinck, et al. 2008; Waksman 2007).

Bioabsorbable Stents

Despite the development and progression of bare metal and drug eluting stents, many concerns still remain regarding their permanent nature and influence on restenosis (Waksman 2007; Sharkawi, et al. 2007). Thus, bioabsorbable stents have emerged as an alternative to permanent metallic stents. Bioabsorbable stents, also described as biodegradable or bioresorbable, are constructed of metallic or polymer materials that degrade over time into substances that can be slowly absorbed by the body (Ormiston, et al. 2008; Erbel, et al. 2007). The specific materials used to create biodegradable stents will be discussed in the following sections. Bioabsorbable stents perform much like other coronary stents except that they are made of biodegradable materials that can be absorbed by the body, allowing them to initially act as supportive scaffolds while slowly disappearing as the vessel heals. The promise behind their technology lies in the fact that they provide a short term vessel scaffold, which can also be used for local drug delivery, and are then absorbed by the body avoiding the long term negative effects of permanent metallic stents such as in-stent restenosis and thrombosis. Currently biodegradable stents are not approved for clinical use in the United States (Ormiston, et al. 2008), however, clinical trials and research into their development are ongoing and release is expected in the near future.

Complex Lesions

Many stents in the market today are designed and indicated for use in simple, straight, short lesions (Fournier, et al. 2005). However, in clinical practice coronary arteries are not straight and uniform. They run along the surface of the heart and are curved, bent, and angulated (Smith, et al. 2001). As a result, coronary stent implantation is being performed in an increasing number of patients with complex lesion characteristics including long, angulated and bifurcated lesions.

Coronary lesions are often extremely long in length and treatment can require multiple overlapping stents (Ruchin, et al. 2008). Therefore, new interlocking stent designs, such as the Custom NX(R) DES System manufactured by XTENT, Inc., are exploring the possibility of deploying stents up to 60 mm in total length (Stella, et al. 2008). It has also been observed that plaque accumulation is related to vessel curvature, with plaque accumulating more rapidly in angulated vessels where wall shear stress is low (Wahle, et al. 2006; Zhu, et al. 2008). Therefore, stents are most often placed in curved positions within the coronary arteries and remain in a bent position throughout their lifetime (Fournier, et al. 2005; Smith, et al. 2001). Many coronary stents, such as the MULTI-LINK manufactured by Abbott Vascular, CYPHER[®] manufactured by Cordis, and TAXUS[®] manufactured by Boston Scientific, claim superior flexibility and conformity which allow the stent to mold to the natural contours of the vessel wall (Serruys and Gershlick 2005; Rieu, et al. 2003). Consequently, accommodation of complex bends and geometries is being taken into greater consideration in overall stent design.

A coronary bifurcation lesion is defined as a narrowing involving both the main vessel and a significant side branch (Louvard, et al. 2008). Bifurcation lesions account for 15-18% of all coronary lesions and represent one of the most complex coronary lesions to treat since the lumen of both the main vessel and side branch are affected and require treatment (Sukhija, Mehta and Sachdeva 2008). As described by the American College of Cardiology/American Heart Association (ACC/AHA) Guidelines for Percutaneous Coronary Intervention a coronary bifurcation occurs when one main vessel branches out into two smaller vessels, one being the continuation of the main vessel, and the other referred to as the side branch (Smith, et al. 2001). Recent advances have allowed physicians to treat bifurcation lesions, where both the main vessel and side branch need to be restored.

Many techniques, with the use of single or multiple stents, have been developed to treat bifurcation lesions (Sukhija, Mehta and Sachdeva 2008; Iakovou, Ge and Colombo 2005). The simplest approach is to stent the main vessel and dilate the side branch through the stent struts. A second approach is to use multiple stents and can be accomplished through a number of different techniques. V-stenting involves the delivery and implantation of two stents, one in each bifurcation branch. The simultaneous kissing technique is similar to V-stenting in that two stents are implanted, one in each branch, with the difference being that the two stents touch and extend into the main branch. Crush, T-stenting and modified T-stenting techniques require two stents to be positioned perpendicular to each other, and while the techniques vary, the resulting stent configurations are identical. Y-stenting involves the delivery and implantation of three stents, one in each bifurcation branch and one in the main branch. Finally, the skirt

technique involves the delivery and implantation of one, two or three stents with the bottom half of the stent dilated to a larger diameter than the top. The bifurcation stenting techniques described are illustrated below in Figure 1.7.

Figure 1.7- Bifurcation stenting techniques. V-stenting (a), simultaneous kissing (b), crush (c), T-stenting and modified T-stenting (d), Y-stenting (e) and skirt (f).

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Most recently, novel stents and delivery systems such as AXXESS™ manufactured by Devax, Inc., PETAL™ manufactured by Boston Scientific, and MULTI-LINK Frontier manufactured by Abbott Vascular have been developed specifically to treat bifurcation lesions (Grube, et al. 2007; Ikeno, Buchbinder and Yeung 2007; Lefèvre, et al. 2005). These stents have the potential to provide physicians with a method to treat complex bifurcation lesions, resulting in better procedural outcomes and

overcoming some of the technical difficulties associated with treating these complex lesions.

In summary, the variation and complexity of coronary artery disease and the advanced capabilities to detect and determine the extent and severity of coronary lesions has led to an increased interest in intravascular device innovation (Kini 2006). With the development of drug eluting and bioabsorbable stents, new drugs and materials are being introduced at an extremely rapid pace (Kraitzer, Kloog and Zilberman 2008). Each new drug and material must be evaluated for biocompatibility and tissue response and the competitive nature of biotechnology has the medical community searching for testing methods and procedures that provide rapid and reliable results.

The blood vessel mimic system has the potential to fulfill this preclinical testing need (Cardinal, et al. 2006). Ideally the BVM system will provide a means to rapidly evaluate human tissue response to varying stent configurations, materials, drugs and coatings as they become available. The safety of drug eluting and bioabsorbable stents, as well as stents designed specifically to treat complex lesions, is still an area of active research (Popma, et al. 2007). However, one thing remains clear- as the understanding of stent and tissue interactions continue to increase, the need to quickly evaluate new pharmacologic agents, configurations and materials will grow, and the need for a model system such as a BVM will be critical for preclinical device testing.

Intravascular Technology

Coronary Stents

The types and construction of coronary stents is an area of active research and stents are constantly undergoing evolution in terms of the materials utilized, geometrical configurations, sizes, and coatings (Sangiorgi, et al. 2007; Hara, et al. 2006; Kandzari, Tchong and Zidar 2002). Stents have evolved from simple coil and slotted tubes to complex configurations with active coatings and advanced features. The current BVM system is a means of evaluating tissue response to varying stent surfaces, coatings, and drugs as well as an evaluation of the physical stents themselves (Cardinal, et al. 2006). The BVM system is designed to test a variety of stent lengths and diameters with varying configurations and coatings, and knowledge of stent design is fundamental for understanding the needs of the system in order to physiologically mimic coronary arteries and induce representative cell response. Therefore, it is necessary to understand the general design and features of stents available in the market today.

Mechanism of Expansion

Stents can be divided according to their mechanism of expansion into two main categories: balloon-expandable and self-expanding (Kraitzer, Kloog and Zilberman 2008; Morton, Crossman and Gunn 2004). Balloon-expandable stents are made from materials that can be plastically deformed through the inflation of a balloon. The stent is mounted on the tip of a balloon catheter and the inflation of the balloon causes the stent to expand.

Once the balloon is deflated the stent remains in its expanded shape inside the vessel. Self-expanding stents, on the other hand, are manufactured in their expanded shape, then compressed and constrained in a delivery system. Upon release from the delivery system they spring back to their manufactured shape. Both balloon-expandable stents and self-expanding stents are readily available in the market; however, balloon-expandable stents are more common. A list of coronary stents and their mechanism of expansion is given in Table 1.1.

Materials

In order to provide effective treatment for coronary artery disease, the ideal stent material must be flexible, fatigue resistant, expandable, corrosion resistant, biocompatible and visible under standard medical procedures (Poncin and Proft 2003). Currently the most widely used material for coronary stents is stainless steel, typically 316L (Mani, et al. 2007; Sangiorg, et al. 2007; Hara, et al. 2006). Stainless steel is both biocompatible and corrosion resistant. In its fully annealed condition, stainless steel is easily deformable and the standard material for balloon-expandable stents. In its full-hard condition it can also exhibit enough elasticity for some self-expanding stent designs.

Although stainless steel is the most commonly used stent material, several other materials are currently used, with many more in development. A list of coronary stents and the material utilized for their construction is given in Table 1.1. The materials most commonly used by stent manufacturers today, after stainless steel, include cobalt chromium alloys, tantalum alloys, nitinol, and various polymers (Mani, et al. 2007;

Morton, Crossman and Gunn 2004). Cobalt chromium is more biocompatible than stainless steel and is being increasingly used by coronary stent manufacturers (Hagemeister, et al. 2005). Cobalt chromium alloys are similar in strength to stainless steel, but have the advantage of being more radiopaque, allowing for the design of thinner struts without compromising visibility (Kandzari, Tchong and Zidar 2002). Nitinol, an alloy of nickel and titanium, is also a common material for coronary stents (Poncet 2005). With its shape memory technology, nitinol is most often used in self-expanding stents.

Pure iron and magnesium alloys are two metals that are under development for use in bioabsorbable coronary stents (Mani, et al. 2007; Erbel, et al. 2007; Waksman 2007; Waksman, et al. 2008). Bioabsorbable metallic stents look promising but their long term biocompatibility still needs to be studied since the elevated local concentration effects of these elements is unknown. In addition to metallic bioabsorbable stent materials, polymers such as poly-L-lactic acid (PLLA), polyglycolic acid (PGA), polylactic-co-glycolic acid (PLGA), poly-D,L-lactic acid (PDLA) and polycaprolactone (PCL) show potential for use in bioabsorbable stents (Ormiston, et al. 2008; Sharkawi, et al. 2007; Waksman 2007). Further research is required, however, in order to better understand the varying tissue responses to these materials.

Stent Name	Manufacturer	Type	Material	Expansion Mechanism	Lesion Type
MULTI-LINK VISION®	Abbott	Bare Metal	Cobalt Chromium	Balloon Expandable	Small Vessel
MULTI-LINK PIXEL®, ZETA®, ULTRA™	Abbott	Bare Metal	Stainless Steel	Balloon Expandable	Small Vessel Large Vessel
MULTI-LINK FRONTIER	Abbott	Bare Metal	Stainless Steel	Balloon Expandable	Bifurcation
Dexamet	Abbott	Drug Eluting	Stainless Steel	Balloon Expandable	Small Vessel
FlexMaster	Abbott	Coated	Stainless Steel	Balloon Expandable	Small Vessel
TriMaxx	Abbott	Bare Metal	Stainless Steel Tantalum	Balloon Expandable	Small Vessel
ZoMaxx	Abbott	Drug Eluting	Stainless Steel Tantalum	Balloon Expandable	Small Vessel
XIENCE V	Abbott	Drug Eluting	Cobalt Chromium	Balloon Expandable	Small Vessel
BVS	Abbott	Bioabsorbable Drug Eluting	Polylactic Acid (PLA)	Balloon Expandable	Small Vessel
TAXUS® Express2	Boston Scientific	Drug Eluting	Stainless Steel	Balloon Expandable	Small Vessel
TAXUS® Petal™	Boston Scientific	Drug Eluting	Platinum Chromium	Balloon Expandable	Bifurcation
Express2	Boston Scientific	Bare Metal	Stainless Steel	Balloon Expandable	Small Vessel
Liberté	Boston Scientific	Bare Metal	Stainless Steel	Balloon Expandable	Small Vessel Large Vessel
PROMUS™	Boston Scientific	Drug Eluting	Cobalt Chromium	Balloon Expandable	Small Vessel
BiodivYsio™	Biocompatibles Cardiovascular	Drug Eluting	Stainless Steel	Balloon Expandable	Small Vessel
Whisper™	Bioabsorbable Therapeutics	Bioabsorbable Drug Eluting	Salicylic Acid	Balloon Expandable	Small Vessel
CYPHER®	Cordis	Drug Eluting	Stainless Steel	Balloon Expandable	Small Vessel
AXXESST™	Devax, Inc.	Drug Eluting	Nitinol	Self-Expanding	Bifurcation
Endeavor	Medtronic	Drug Eluting	Cobalt Chromium	Balloon Expandable	Small Vessel
Blazer	OrbusNeich	Bare Metal	Cobalt Chromium	Balloon Expandable	Small Vessel
R-Stent	OrbusNeich	Bare Metal	Stainless Steel	Balloon Expandable	Small Vessel
Carbostent™	Sorin Biomedica	Coated	Cobalt Chromium	Balloon Expandable	Small Vessel
Custom NX(R)	Xtent	Drug Eluting	Cobalt Chromium	Balloon Expandable	Small Vessel

Table 1.1- Coronary stent type, material and mechanism of expansion.

Data gathered from references cited throughout text.

Geometry and Configuration

Coronary stents come in a variety of different geometrical configurations. Early stent designs were generally classified as either slotted tube or coil geometries (Morton, Crossman and Gunn 2004). Slotted tube designs were simply metal tubes in which slots or sections were cut out, leaving open patterns. Coiled stents, on the other hand, were made of wire or sheet metal fashioned into tube-like structures. Early coiled and slotted tube stents are shown below in Figure 1.8.

Figure 1.8- Early coronary stent designs. Coiled (a) and slotted tube (b) geometries.

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While slotted tube designs had excellent radial strength, they lacked flexibility; the opposite occurred for coil designs (Sangiorgi, et al. 2007; Morton, Crossman and Gunn 2004). The resulting evolution of stent design gave way to the development of a large variety of stent geometries and configurations including coils, wire mesh, slotted tubes, corrugated, and interconnected rings. Currently, the majority of stents in clinical practice are classified as slotted tube or cellular designs which are either laser-cut from a metal tube or composed of interconnected wire rings (Morton, Crossman and Gunn 2004; Lau, et al. 2004). Common stent geometries are shown below in Figure 1.9.

Figure 1.9- Current coronary stent designs. Sorin Carbostent (a), Medtronic beStent 2 (b), Cordis BxVelocity (c), and Boston Scientific Multilink Tetra (d).

(Permission granted to reprint image from Colombo, Stankovic and Moses 2002 published in Journal of the American College of Cardiology by Elsevier Limited.; see Appendix G)

As stated in the previous section, novel stents have been developed specifically to treat bifurcation lesions (Ikeno, Buchbinder and Yeung 2007). These stents have varying configurations including branches, holes, and other geometrical features that allow for stenting of both the main branch and side branch. Several of the new bifurcation stent designs employ an innovative Y-shaped or T-shaped geometry in order to match the anatomy of lesions that form at the junctions of coronary arteries. The PETAL™ bifurcation stent manufactured by Boston Scientific is shown below in Figure 1.10.

Figure 1.10- Bifurcation stent design. PETAL™ bifurcation stent manufactured by Boston Scientific.

(Permission granted to reprint image from Ikeno, Buchbinder and Yeung 2007 published in Cardiovascular Revascularization Medicine by Elsevier Limited.; see Appendix G)

Different stent designs yield varying tissue responses following implantation (Hara, et al. 2006) and research concerning the relation of stent geometry, strut thickness and in-stent restenosis is ongoing. These design variations, as well as the desire to better understand the impact of stent characteristics on tissue response, result in a need for preclinical testing environments that support a range of stent shapes, geometries and configurations.

Diameter and Length

Coronary stents come in varying diameters and lengths (Kraitzer, Kloog and Zilberman 2008). A list of coronary stents along with their diameters and lengths is given in Table 1.2. The most common diameters available include: 2.00 mm, 2.25 mm 2.50 mm, 2.75 mm, 3.00 mm, 3.50 mm, 4.00 mm, 4.50 mm and 5.00 mm. The most common lengths include: 8 mm, 12 mm, 13 mm, 15 mm, 18 mm, 23 mm, 28 mm, 33 mm, and 38 mm. Some of the most recent designs, such as the Custom NX(R) DES System manufactured by XTENT, Inc., allow physicians to choose the appropriate stent length while inside the artery at the site of the lesion (Stella, et al. 2008). This variation results in a need for preclinical testing environments that support a range of stent sizes, ideally 2.0-5.0 mm diameters and 8-38 mm lengths (Kandzari, Tchong and Zidar 2002; Kraitzer, Kloog and Zilberman 2008), in order to fit the variations of coronary arteries observed in clinical practice and the stents currently available in the market.

Coatings

The surface properties of coronary stents significantly influence tissue response (Mani, et al. 2007). For this reason, coatings are often applied to metallic stents in order to change their surface properties and improve biocompatibility. These modifications can also inhibit neointimal proliferation and thrombosis, which can in turn reduce the rate of restenosis (Lau, et al. 2004). A list of coronary stents and the type of coating applied is given in Table 1.2.

The main advantage of a stent coating is the combination of mechanical stability and increased biocompatibility (Mani, et al. 2007). Coatings are a film of material bonded to the metallic surface of the stent, or embedded within the porous structure, and can be either passive or active. Passive coatings are inert and non-reactive in the body, while active coatings induce a biological response (Karoussos, et al. 2002). Many considerations must be taken into account when applying a stent coating including the biocompatibility, long-term effects, and material properties. In addition, it has been shown that stent coatings should not substantially enhance strut thickness because this promotes neointimal proliferation (Wieneke, et al. 2003).

Organic coatings include both biodegradable polymers, including PLLA, PGA, PLGA, PDLA, and PCL as discussed previously, and non-biodegradable polymers. Polymer coatings have been proposed to improve the biocompatibility of metallic stents and to serve as a matrix for drug delivery (Karoussos, et al. 2002). Phosphorylcholine (PC) based polymer coatings, unlike many other polymer coatings, do not provoke excessive inflammatory or adverse reactions and provide a useful drug delivery platform,

especially for controlled release of drugs (Mani, et al. 2007; Waksman 2002). Other non-biodegradable polymers for the potential use in controlled drug release include hyaluronic acid (HA), polyethylene-co-vinyl acetate (PEVA) and poly n-butyl methacrylate (PBMA).

There are many inorganic coating materials which are suitable as stents coatings. Gold, silicon carbide, aluminum oxide and diamond-like carbon are some of the most common (Mani, et al. 2007). Gold is highly radiopaque and has been shown to be very biocompatible and significantly reduce neointimal proliferation and thrombosis. However, clinical studies have shown that gold coated stents increase the risk of restenosis (Karoussos, et al. 2002; vom Dahl, et al. 2002). Silicon carbide is a passive stent coating, which has been shown to decrease thrombosis and increase biocompatibility. Even so, silicon carbide coatings do not significantly reduce restenosis and do not have any known clinical advantage over other stent coatings (Hara, et al. 2006). Porous ceramic coatings have become very promising for use as drug delivery platforms. The most recent development is a ceramic aluminum oxide coating with a unique nanoporous surface (Kang, et al. 2007; Wieneke, et al. 2003). Inorganic ceramic nanoporous aluminum oxide coatings have been shown to reduce neointimal proliferation and are specifically designed for incorporation of anti-restenosis drugs, which will be discussed in detail in the following sections. Lastly, carbon coated stents are coated with pure diamond-like carbon which is characterized by a polycrystalline structure and shown to improve biocompatibility (Mani, et al. 2007; Karoussos, et al. 2002). Currently, however, carbon coatings are not common in clinical use.

Drugs

The ideal agent for incorporation into drug eluting stents should have potent anti-restenosis effects while preserving vascular healing (Kraitzer, Kloog and Zilberman 2008). Anti-inflammatory, anti-thrombogenic, anti-proliferative, and immunosuppressive agents which interfere with early stages of the cell cycle are currently used in coronary stent designs to prevent or slow the progress of in-stent restenosis. Each of these agents inhibits one or more of the biological processes involved in restenosis and have revolutionized the treatment of coronary artery disease (Waksman 2002). Table 1.2 provides a list of coronary stents and the drugs incorporated into their designs.

Paclitaxel, synthetically produced as Taxol, is a potent inhibitor of cell proliferation and prevents mitosis of cells during the G2-M phase of the cell cycle (Hara, et al. 2006). The cell cycle consists of five basic steps: mitosis (M), gap phase 1 (G1), dormancy (G0), synthesis (S) and gap phase 2 (G2). G2-M is the transition in the cell cycle from state G2, where the cell produces enough proteins to divide, to state M, where the cell divides into two daughter cells. Paclitaxel acts by binding to microtubules during the G2 phase, thus preventing mitosis and cell replication. As a result, it inhibits smooth muscle cell proliferation and smooth muscle cell migration and has been shown to reduce inflammation (Deconinck, et al. 2008). Sirolimus, also known as rapamycin, its analogues zotarolimus and everolimus, and ABT-578 which is a synthetic analogue of rapamycin, block cell cycle progression at the G1-S transition (Kraitzer, Kloog and Zilberman 2008). G1-S is the transition in the cell cycle between phase G1, where the cell increases in size while producing proteins and RNA, to phase S when DNA

replication occurs. As a result, it inhibits smooth muscle cell proliferation as well as T-cell and B-cell proliferation. Sirolimus and its derivatives are also known to prevent smooth muscle cell migration and reduce inflammation (Deconinck, et al. 2008; Mani, et al. 2007). An illustration of the cell cycle is shown below in Figure 1.11 identifying the cell cycle transition phase inhibited by both paclitaxel and sirolimus.

Figure 1.11- Cell Cycle. Sirolimus blocks cell cycle progression at the G1-S transition while paclitaxel prevents mitosis of cells during the G2-M transition.

(Permission granted to reprint image from Hara, et al. 2006 published in Advanced Drug Delivery Reviews by Elsevier Limited.; see Appendix G)

Other common agents for incorporation into drug eluting stents include tacrolimus (FK506), dexamethasone, and heparin (Deconinck, et al. 2008; Kraitzer, Kloog and Zilberman 2008). Tacrolimus and dexamethasone are both cellular proliferation inhibitors. Tacrolimus prevents the proliferation of T-cells, lymphocytes and smooth

muscle cells. Dexamethasone is a well established anti-inflammatory agent and inhibits the proliferation of fibroblasts, smooth muscle cells and macrophages. Heparin, an anticoagulant, reduces leukocyte and platelet adhesion and protects against stent thrombosis (Mani, et al. 2007; Hara, et al. 2006). It also prohibits smooth muscle cell migration and proliferation.

Localized drug delivery using stents as the drug delivery platform is advantageous since the drug is release at the precise location and at the time of vessel injury (Kraitzer, Kloog and Zilberman 2008). In addition, higher local concentrations are achievable with little risk of system toxicity. Currently there are three different approaches to binding drugs to coronary stents: drug binding by means of a polymer on the stent surface, drug binding aided by an inorganic stent coating, or direct drug binding to the stent surface without coating (Acharya and Park 2006). Drugs are most often either chemically bonded to the surface of the stent or trapped within a polymer film or porous ceramic that allows controlled drug release (Kandzari, Tchong and Zidar 2002). Drug release in biodegradable coatings is achieved through disintegration of the biodegradable polymer of the stent, while non-biodegradable coatings release drugs by allowing the drugs to diffuse through a porous matrix.

Stent Name	Manufacturer	Diameter (mm)	Length (mm)	Coating	Drug
MULTI-LINK VISION®	Abbott	2.0,2.25,2.5 2.75,3.0,3.5,4.0	8,12,15 18,23,28	None	None
MULTI-LINK PIXEL®, ZETA®, ULTRA™	Abbott	2.0,2.25,2.5 2.75,3.0,3.5 4.0,4.5, 5.0	8,13,15,18 23,28,33,38	None	None
MULTI-LINK FRONTIER	Abbott	2.5,3.0,3.5,4.0	18	None	None
Dexamet	Abbott	2.0,2.25,2.5 2.75,3.0,3.5,4.0	7,8,10,11,15 18,22,28	Phosphorylcholine (PC)	Dexamethasone
FlexMaster	Abbott	2.5,2.75 3.0,3.5,4.0	9,12,16 19,23,26	None	Heparin
TriMaxx	Abbott	2.25,2.5,2.75 3.0,3.5,4.0	8,13,15 18,23,28	None	None
ZoMaxx	Abbott	NA	NA	Phosphorylcholine (PC)	Zotarolimus
XIENCE V	Abbott	2.5,2.75 3.0,3.5,4.0	8,12,15 18,23,28	Polyvinylidene Fluoride (PVDF) Hexafluoropropylene (HFP)	Everolimus
BVS	Abbott	NA	NA	None	Everolimus
TAXUS® Express2	Boston Scientific	2.5,2.75 3.0,3.5	8,12,16,20 24,28,32	Polystyrene-b-isobutylene-b-styrene	Paclitaxel
TAXUS® Petal™	Boston Scientific	NA	NA	Polystyrene-b-isobutylene-b-styrene	Paclitaxel
Express2	Boston Scientific	2.5, 2.75	8,12,16 20,24	None	None
Liberté	Boston Scientific	2.75,3.0,3.5 4.0,4.5,5.0	8,12,16,20 24,28,32	None	None
PROMUS™	Boston Scientific	2.5,2.75 3.0,3.5,4.0	8,12,15 18,23,28	Polyvinylidene Fluoride (PVDF) Hexafluoropropylene (HFP)	Everolimus
BiodivYsio™	Biocompatibles Cardiovascular	NA	NA	Phosphorylcholine (PC)	Dexamethasone
Whisper™	Bioabsorbable Therapeutics	NA	NA	None	Sirolimus Salicylic Acid
Conor Medstent™	Conor Medsystems	NA	NA	Poly(lactide-co-glycolic Acid (PLGA)	Paclitaxel
CYPHER®	Cordis	2.5,2.75 3.0,3.5	8,13,18 23,28,33	Polyethylene-co-vinyl Acetate (PEVA) Poly-n-butyl Methacrylate (PBMA)	Sirolimus
ACHIEVE™	Cook	NA	NA	None	Paclitaxel
AXXESS™	Devax, Inc.	NA	NA	Poly(lactic Acid (PLA)	Biolimus A9®
Endeavor	Medtronic	NA	NA	Phosphorylcholine (PC)	Zotarolimus
Blazer	OrbusNeich	2.5,2.75 3.0,3.5,4.0	9,13,15 18,23,28,33	None	None
R-Stent	OrbusNeich	2.5,2.75 3.0,3.5,4.0	9,13,15 18,23,28,33	None	None
Janus Carbostent™	Sorin Biomedica	NA	NA	Carbon	Tacrolimus
Custom NX(R)	Xtent	NA	36,60	Poly(lactic Acid (PLA)	Biolimus A9®

Table 1.2- Coronary stent diameters, lengths, coatings and drugs.

Data gathered from references cited throughout text.

There are many different stent coatings and drugs being researched today and only the most promising were addressed in this section. Those in clinical use at the present time are being perfected and many new and different coatings and drugs will be developed in the future. Tables 1.1 and 1.2 illustrate the various combinations of geometries, sizes, coatings, and drugs currently available in the market as well as those being designed for future clinical use. Many of these stents are currently undergoing clinical trials and are likely to be released in the near future. Others are still in the initial design phases and in need of a preclinical testing environment such as the BVM system.

Delivery Systems

In addition to the advancements in coronary stent design, the method of delivering these therapies has also improved. These improvements have made percutaneous coronary interventional procedures easier to perform and have reduced the amount of time for a single procedure. Over-the-wire delivery systems represented the first significant innovation in PCI procedures (Abrams, Baum and Pentecost 2006; Simpson, et al. 1982). Over-the-wire delivery systems allow manipulation of the guidewire independently from the balloon catheter. Rapid exchange, or monorail, delivery systems represent the next advancement and were developed to simplify the exchange of catheters by allowing a much shorter length of guidewire to be used. Rapid exchange delivery systems allow a single operator to manage both the catheter and the guidewire during a PCI procedure (Bonnett, et al. 2004).

Stent delivery and implantation in complex coronary geometries is a challenge for conventional delivery systems. It is often difficult to maneuver 6 French catheters, the current standard for most stent delivery devices, through tortuous vessel geometries (Saeed, Banerjee and Brilakis 2008). The French catheter scale is commonly used to measure the outer diameter of cylindrical medical instruments, with one French equivalent to 0.013 inches. As a result, a novel 4 French balloon catheter was designed for the treatment of a variety of coronary artery lesions and provides a less invasive method of treatment while improving procedural outcomes (Takeshita, et al. 2008). The CardioMind Sparrow™ coronary stent delivery system manufactured by CardioMind is another low profile delivery device consisting of a self-expanding nitinol stent loaded into a 0.014 inch guidewire platform with the ability to travel through tortuous geometries (Yamasaki, et al. 2008; Abizaid, et al. 2007).

In addition to tortuous vessel geometries and the need for lower profile and more flexible delivery systems, complex coronary lesions often require high-pressure balloon inflation for complete stent and lumen expansion. The use of high-pressure balloon inflation has been associated with an increased incidence of restenosis due to injury caused by balloon inflation (Oesterle, et al. 1998). Therefore, novel low-pressure dilation balloon catheters, such as the FX miniRAIL manufactured by X Technologies, are being explored as a method to reduce the balloon pressure required for stent expansion in complex coronary lesions (Ischinger, Solar and Hitzke 2003).

Percutaneous coronary intervention in complex coronary geometries is difficult and the increased complexity results in reduced success rates (Saeed, Banerjee and

Brilakis 2008). Therefore, novel delivery systems, smaller catheters, lower profile balloons and other devices have been created to overcome the challenges associated with tortuous anatomies. Stent delivery is an area of active research and a living model system for testing and evaluating these new delivery devices would be ideal for development and progression of the field.

Preclinical Device Testing

Preclinical device testing is a crucial step in the process of developing and releasing new medical devices to the market. The objective of preclinical testing is to provide preliminary evidence of product safety and efficacy in order to support the initiation of human clinical trials (Wall and Shani 2008). Preclinical testing typically begins using models and methods that are simple and cost-effective in order to identify potential failures as early in the development process as possible, and then progresses to models with increasing complexity. Preclinical intravascular device testing is currently performed using laboratory models, *in vitro* cellular models, and *in vivo* animal models and provides critical information about cellular and tissue responses as well as device success.

Laboratory Models

One of the first steps in preclinical intravascular device testing involves the use of laboratory models. Prior to determining the cellular response to a device research must be performed in order to verify device functionality. Laboratory models are often used to physically mimic the geometry of coronary arteries and allow a large number of device designs to be evaluated quickly and inexpensively. For example, synthetic models in which vessel shaped troughs are bored through plastic blocks are often constructed for preclinical testing of stents in order to simulate device expansion and delivery within a coronary vessel (Ormiston, et al. 1999). Other models use flexible tubing to mimic the vascular structure of the coronary arteries and foam-like material to represent and mimic

the presence of a lesion (Connolley, et al. 2007). These types of synthetic model approaches allow stents to be deployed and evaluated in coronary geometries; however, no cellular lining exists to provide physiological response evaluations.

It is important to remember that stent implantation is a mechanical intervention and the engineering aspects and mechanical performance of the stent design must be evaluated. Methods for identifying mechanical complications enable preclinical screening of stent designs, which reduces the amount of animal testing required and provides a method for evaluating new stent designs and stenting techniques prior to animal and human clinical trials.

In Vitro Cellular Models

Following functional and mechanical testing in a laboratory model, *in vitro* cellular models are the next step in preclinical intravascular device testing. Although a more complex dynamic system exists *in vivo*, a common preclinical evaluation method involves culturing endothelial cells *in vitro* in order to study their response to new drugs, materials, and devices (Cenni, et al. 1999). These cells can be maintained as two-dimensional monolayers in flasks or dishes, or cultured in three-dimension adhered to scaffolds or suspended in solution (Bokhari, et al. 2007; Cheng, Lai and Kisaalita 2008; Cui, et al. 2007; Sun, et al. 2006). Either of these cellular models can then be exposed to samples of new materials or medical devices in order to assess responses such as cell proliferation, cell migration, and cell death. Although *in vitro* cell cultures do not reflect the complexity of a living system, they provide important insights into cellular response

and are critical in the effort to minimize the time and cost associated with *in vivo* animal and human studies.

In Vivo Animal Models

Currently most preclinical biocompatibility and tissue response testing is done on animals in outside laboratories, which is both time consuming and expensive. Animal studies, however, are a valuable tool and much of what is known about restenosis and the healing response after stent implantation has been gathered from the study of both healthy and atherosclerotic animal models (Touchard and Schwartz 2006; Schwartz, Chronos and Virmani 2004; Wieneke, et al. 1999).

The ideal animal model for preclinical intravascular device testing should be readily available, inexpensive, and easy to handle. More importantly, it should closely mimic the human pathophysiology of neointimal proliferation and remodeling (Russell and Proctor 2006; Kantor, et al. 1999). Current animal models for preclinical intravascular device testing include mice, rats, rabbits, pigs, dogs, and primates (Russell and Proctor 2006; Touchard and Schwartz 2006; Schwartz, Chronos and Virmani 2004).

The Apo E-deficient mouse model has become the most widely used animal model in atherosclerosis research (Desai, Zhao and Warren 2008; Fazio and Linton 2001). Apo E-deficient mice have been genetically altered so that they naturally develop high cholesterol, leading to atherosclerosis and the development of coronary lesions and blockages much like those found in human coronary arteries (Russell and Proctor 2006).

The rat carotid artery and the rabbit iliac model are the most commonly used animal models for testing intravascular devices targeted at preventing restenosis. Rats and rabbits are small and relatively inexpensive, providing a fast and reliable animal model for preclinical testing. One disadvantage is that rats and rabbits are highly resistant to the development of atherosclerosis, necessitating the use of either a high cholesterol diet or vessel injury to mimic coronary lesions (Schwartz, Chronos and Virmani 2004; Wieneke, et al. 1999). However, even healthy rat and rabbit models provide important information regarding the vascular healing response that occurs after device implantation.

In addition to rats and rabbits, the pig has become a widely used species for assessing treatments of restenosis because its coronary anatomy and physiology are similar to those of humans and are susceptible to atherosclerosis, developing thick neointimal lesions in response to injury (Russell and Proctor 2006; Schwartz, Chronos and Virmani 2004). Large animals, such as dogs and primates, are also useful models but are not commonly used due to high cost, ethical considerations, and long life spans.

Although preclinical animal studies are accepted as a means to predict the probability of device safety and effectiveness in humans, no animal model can provide complete insight into the human healing response following stent implantation (Wall and Shani 2008; Fazio and Linton 2001; Wieneke, et al. 1999). The need exists for preclinical testing using live human cells and tissue. Preclinical testing using human cells would greatly increase the medical community's understanding of tissue response to a specific device prior to animal and patient testing and prior to device release. Presently, it is difficult to simulate device and tissue interaction and research still needs to be done to

fully understand tissue behavior and the *in vivo* interaction that occurs between device and tissue.

Blood Vessel Mimic

Previous work has demonstrated that a 3-dimensional *in vitro* tissue engineered blood vessel mimic (BVM) can be created and maintained in a bioreactor system to serve as a potentially ideal environment for coronary stent implantation and testing prior to animal and clinical studies (Cardinal, et al. 2006). The *in vitro* blood vessel mimic system can allow initial preclinical testing to be performed on a large number of coating combinations and stent configurations in order to direct animal and clinical studies toward the most promising devices. The major advantage of the *in vitro* blood vessel mimic system is its potential for consistent high-throughput evaluation of cellular response to advancing intravascular technologies.

The *in vitro* blood vessel mimic design and concept is based upon existing techniques for tissue engineered vascular grafts and bioreactor systems. Human microvascular endothelial cells isolated from adipose tissue are pressure seeded onto tubular expanded polytetrafluoroethylene (ePTFE) scaffolds and cultivated in bioreactor systems under flow to form a “blood vessel mimic”. Results demonstrate that a thin, confluent cellular lining on the inner lumen of the ePTFE scaffold can be created *in vitro*. Stent deployment within the blood vessel mimic is also possible and after being cultivated in the bioreactor system for several days a thin lining of cells forms over the stent surface.

The BVM system described above (Cardinal, et al. 2006) consists of a custom built horizontal rectangular vessel chamber that houses an ePTFE scaffold. An individual BVM chamber is shown below in Figure 1.12. Following vessel cultivation and growth within the chamber, stents or other intravascular technologies can be evaluated and tested.

Figure 1.12- Individual BVM chamber.

The BVM system provides an intermediate testing environment for assessing human cellular responses to intravascular stents in a manner that combines the advantages of both *in vitro* and *in vivo* approaches. The blood vessel mimic system utilizes human cells, which provide the potential for more predictive preclinical results than animal models alone can provide. It is important to note that the blood vessel mimic system is not a substitute or replacement for animal models. The overall goal of the BVM system is to provide a more relevant *in vitro* tissue model that can be used for the preclinical evaluation of new intravascular devices.

Summary and Aims of the Thesis

Coronary artery disease affects millions of people and the ability to detect and treat the disease is advancing at a rapid rate. Intravascular device technology is the focus of many medical device manufacturers and with coronary stent implantation being performed in an increasing number of patients a number of new stent designs have been introduced to the market, resulting in the need for improved preclinical testing methods.

A blood vessel mimic (BVM) system has previously been established and its feasibility for the initial testing of newly emerging intravascular technology has been demonstrated. The current BVM system has been used successfully to support the growth of tissue engineered *in vitro* blood vessel mimics, and its components and usefulness have been summarized. With that said, there are limitations that exist with the original design and the focus of this thesis is to both improve and expand upon the original model. The goal of this thesis is to develop a BVM system which has the advantages of simplicity and ease of assembly, as well as the ability to accommodate varying sizes of vessel scaffolds and to expand the BVM concept to an area that currently has not been addressed, such as long, angulated and bifurcation lesions, all while maintaining the needs of a tissue engineering bioreactor system.

The general hypothesis driving this thesis project was that design changes could be made and new configurations implemented to the BVM system that would improve function and usability for preclinical device testing. In order to test this hypothesis, research was conducted based on two specific aims. The first aim was to develop a more ideal BVM system. The specific hypothesis in this case was that a BVM chamber could

be created to accommodate a wider range of stent lengths and diameters, while allowing for easy graft insertion and seal-ability. The second aim was to develop next generation BVM systems, focused on future needs and technology. The hypothesis in this case was that with emerging technology the ability to test stents specifically for bifurcation lesions, as well as in long and angulated vessels, will be needed and is possible within the BVM system. The prototypes developed and the findings from constructed models are presented and discussed in the following chapters.

2. INITIAL DESIGN OF AN IDEAL BVM

Introduction

A bioreactor is a system in which conditions are closely controlled in order to induce a specific behavior in living cells or tissue (Martin, Wendt and Heberer 2004; Korossis, et al. 2005). Design and construction of bioreactors are very important in tissue engineering research, because they directly provide the *in vitro* environment for tissue growth (Chen and Hu 2006). A bioreactor attempts to mimic physiological conditions in order to maintain and encourage cell growth. Bioreactor technologies intended for tissue engineering can be used to grow functional cells and tissues for transplantation and for controlled *in vitro* studies. It is the second application on which this thesis is focused: the use of bioreactors for controlled *in vitro* studies.

The primary objectives of tissue engineering bioreactors are to establish spatially uniform cell distributions on three dimensional scaffolds, to maintain desired concentrations of gases and nutrients in the culture medium, and to expose developing tissue to appropriate physical stimuli (Korossis, et al. 2005). The requirements of a bioreactor vary depending on the dimensions, complexity, and physiological environment of the tissue to be engineered. Most importantly, however, the bioreactor has to be able to operate over sustained periods of time under aseptic conditions so that cells are able to grow and function (Chen and Hu 2006). The overall goal is to create a system that reliably and reproducibly supports the growth of functional living tissue (Martin, Wendt and Heberer 2004; Ratcliffe and Niklason 2002).

The previously developed, original BVM system (Cardinal, et al. 2006) consists of a custom built horizontal rectangular vessel chamber with three ports: a luminal inlet, luminal outlet, and extra luminal outlet. Both outlets lead to a two-port media reservoir, with external side clamps controlling the flow path. The scaffold is attached to two barbed fittings which are inserted into the luminal inlet and luminal outlet ports located within the vessel chamber. Adjustments to length are made by pulling or pushing the tubing in or out of the chamber. The top of the chamber is optically clear allowing for easy visualization of the scaffold, media and stent placement. The chamber itself is sealed using rubber o-rings and clamped together by tightening four screws. Flow is created using a peristaltic pump, with media entering the luminal inlet and exiting through the luminal outlet and extra luminal outlet. The system can be sterilized using ethylene oxide (EO) gas. Although advantages of this original system exist, it would be desirable to improve upon this design in order to create a more ideal BVM system.

One common disadvantage among many bioreactor systems is the amount of difficulty experienced when installing or removing scaffolds (Singh, et al. 2007), and this is true for the original BVM system. This is often a challenge in itself as sterility needs to be maintained at all times. Easy handling and assembly also lowers the risk of contamination (Fisher, Mikos and Bronzino 2007). A more user friendly design is one of the primary needs of the BVM system. Easy scaffold insertion, without the need for bending or twisting, along with a method to quickly and simply seal the vessel chamber without the need for tools, would greatly improve the usability of the BVM system.

A second need for the BVM system is the ability to accommodate a wider range of stent sizes. As discussed in detail in the introduction to this thesis, stents range in shape and size, and thus a BVM system would ideally accommodate 2.0-5.0 mm diameters and 8-38 mm lengths (Kandzari, Tcheng and Zidar 2002; Kraitzer, Kloog and Zilberman 2008), in order to fit the variations of coronary arteries observed in clinical practice and the stents currently available in the market.

In addition, ideally the BVM system would be easy to manufacture and utilize materials and components that are readily available and cost-effective so that scale-up is possible (Fisher, Mikos and Bronzino 2007; Martin, Wendt and Heberer 2004). Other considerations in design of the BVM system include sterilization method, incubator configuration, aseptic change of culture media, tissue monitoring, and reproducibility (Fisher, Mikos and Bronzino 2007). The aim of the work in this chapter was to improve upon the original BVM system by developing a new, more ideal design, with the hypothesis being that a simpler, easier to use and more variable design could be created and implemented for preclinical device testing. The specific design criteria to be met are summarized below:

- An overall user friendly design so that assembly is simple and straightforward
- Easy scaffold insertion and removal without the need for bending or twisting
- Easy to seal leak-proof chamber for quick and sterile assembly
- Adjustable scaffold length and diameter for greater testing variability
- Sterilizable by ethylene oxide (EO) gas
- Optically clear chamber for easy visibility of scaffold, media and stent placement

- Easy scale-up so that multiple chambers can be configured in the incubator
- Low cost
- Easy to manufacture without the need for adhesive or special tooling
- Use of materials and components that are readily available and easy to obtain
- Use of biocompatible materials so that cells are able to grow and function

Initial Design Models and Concepts

Initial design ideas and concepts were drawn and modeled. These designs were free flowing ideas based only on the known limitations, needs and design criteria to be met for the new BVM system. Ideas were recorded or drawn in a lab notebook over a time period of several days before any evaluations were made. Drawings were then turned into basic 3D models using SolidWorks (SolidWorks Student Design Kit 2008) for visualization and comparison. These designs were recorded without regard to feasibility of design, availability of components, cost or any other idea limiting factors. It should be noted that only the luminal inlet and luminal outlet were modeled, since location of the extra luminal outlet was not critical to the basic design. In addition, tubing attachment, connections and fittings were not addressed in these initial designs as the goal was only to develop a basic model. The five initial design ideas that were developed are modeled and described below.

Prior to the building of any prototypes the initial design ideas and concepts were reviewed and evaluated based on the following criteria: user friendly design, seal-ability, availability of materials and components, adjustability, and overall cost. Each model was examined to first determine if it could actually be built and function, and if so, whether the components were readily available or easy to create. Finally, even if the design could be built and parts were available, consideration was taken into design complexity, manufacturability and overall cost. The evaluation of each initial design is presented below following the design model and description.

Initial Design 1

Description: The first initial design idea consisted of clear plastic rectangular top and bottom sections that meet at the center and snap together. The two sections could be separate pieces or possibly held together by a hinge. The entire midsection would contain a rubber o-ring and when snapped together the top and bottom sections would create a leak-proof seal. Two centered holes would be made on opposite ends of the box, resulting in half circle indentations on both the top and bottom sections. The two holes on either end of the box would also contain a rubber seal and when snapped together they would encompass the scaffold and tubing and create a tight leak-proof seal around the tubing. During set-up, the scaffold and tubing would be placed along the center of the bottom section, within the half circle indentations, prior to placement of the top section. Initial Design 1 is modeled in Figures 2.1 and 2.2 below.

Figure 2.1- Exploded 3D model of Initial Design 1. An exploded view shows the overall design concept with the top and bottom sections separated. The scaffold and tubing is set across the bottom section and placed within the half circle indentations.

Figure 2.2- Assembled 3D model of Initial Design 1. An assembled view shows the overall design concept with the top and bottom sections snapped and sealed together. The scaffold and tubing is encompassed between the top and bottom sections, with the half circle indentations sealed around the tubing.

Evaluation: The biggest advantage of this design is that the snapping top and bottom sections would be easy to assemble and use. This design would be easy to open, close and put together. The box design would be easy to configure in an incubator and is stackable. Scaffold placement would be simple, as it would lie across the bottom section within the half circle indentations. The scaffold and tubing could be set in place without the need for stretching or bending. Clear plastic would allow for visibility and could be sterilized using ethylene oxide (EO) gas. The scaffold length would be somewhat variable, but limited by the chamber length. The limiting factor of this design is sealability. The entire midsection must seal together and the half circle indentations must create a leak-proof seal around the tubing. Manufacture of this type of seal design would be complicated, expensive and require adhesive and custom made parts. The major disadvantage of this design was that no container could be found with this configuration

and creating a functioning seal would be challenging. The overall evaluation of Initial Design 1 is summarized below:

- User friendly design: Yes- chamber would be easy to assemble and scaffold would be easy to insert
- Seal-ability: Difficult- it would be hard to design and ensure a complete leak-proof seal across the tubing and along all edges
- Availability of materials and components: Custom- some components would need to be custom built and designed
- Adjustability: Limited- longest scaffold would be limited by chamber length
- Cost: Moderate to High- due to custom parts and complex seal design

Initial Design 2

Description: The second initial design idea consisted of two clear plastic cylindrical sections that meet at the center and slide together, encompassing the scaffold and tubing. The two sections would be separate pieces with the inside diameter of the larger section matching the outside diameter of the smaller section. The open ends of the cylindrical sections would contain rubber o-rings and when slid together the two sections would create a leak-proof seal. The resulting seal would function both in a stationary position and when the two sections are slid together or apart. Two centered holes would be made on opposite ends of the cylinder, with the tubing and scaffold placed within the center. During set-up, the scaffold would be inserted into the tubing, prior to closure of the two sections. Initial Design 2 is modeled in Figures 2.3 and 2.4 below.

Figure 2.3- Exploded 3D model of Initial Design 2. An exploded view shows the overall design concept with the two cylindrical sections separated. The scaffold and tubing is placed within the center.

Figure 2.4- Assembled 3D model of Initial Design 2. An assembled view shows the overall design concept with the two cylindrical sections slid and sealed together. The scaffold and tubing is encompassed within the two cylindrical sections.

Evaluation: The biggest advantage of this design is that the length of the chamber would be easily adjustable. The two sliding sections would allow for variable scaffold length with precise adjustment of chamber length depending on the scaffold size used. The sliding cylindrical sections would be easy to assemble and scaffold placement would be simple. Clear plastic would allow for visibility and could be sterilized using ethylene oxide (EO) gas. Again, the overall limiting factor of this design is seal-ability. This design would need circular o-ring seals on the cylindrical sections, and the seals would need to hold and remain leak-proof while sliding. Manufacture of this type of seal design would be complicated, expensive and most likely wear out over time due to sliding. This design would be difficult to build even though the materials are available. Usability is also a concern. A cylindrical shape would be challenging to work with and place in the incubator as it would roll and is not stackable. This design would also be difficult to configure in regards to tubing adjustability, as the tubing would also need to slide and

adjust as the chamber slides. The overall evaluation of Initial Design 2 is summarized below:

- User friendly design: Somewhat- chamber would be easy to assemble and scaffold would be easy to insert, but chamber would roll on a flat surface
- Seal-ability: Difficult- it would be hard to design and ensure a complete leak-proof sliding seal that would not wear over time
- Availability of materials and components: Custom- some components would need to be custom built and designed
- Adjustability: High- chamber length can be easily adjusted to scaffold length
- Cost: Moderate to High- due to custom parts and complex seal design

Initial Design 3

Description: The third initial design idea consisted of a clear plastic cylindrical center section with clear plastic adjustable corrugated tubing on both ends. The corrugated tubing sections could be pushed and pulled to change the overall length of the chamber. The corrugated tubing would be permanently attached and sealed to the center section with one end completely closed. The other end of the corrugated tubing would have a threaded or snapping leak-proof cap to create an opening for scaffold placement. Two centered holes would be made, one on the closed end of the cylinder and one on the cap, and the tubing and scaffold would be placed through the center. During set-up, the scaffold would be inserted into the tubing, prior to closure of the cap. Initial Design 3 is modeled in Figures 2.5 and 2.6 below.

Figure 2.5- Side view 3D model of Initial Design 3. A side view shows the overall design concept with the corrugated tubing sections attached to the center section. The scaffold and tubing is placed within the center.

Figure 2.6- Assembled 3D model of Initial Design 3. An assembled view shows the overall design concept with the corrugated tubing sections and center sealed together. The scaffold and tubing is encompassed within the chamber.

Evaluation: The biggest advantage of this design is that the length of the chamber would be easily adjustable. The corrugated tubing could be pushed and pulled and would allow for variable scaffold length with precise adjustment of chamber length depending on the scaffold size used. The snapping cap would be easy to assemble and seal. Clear plastic would allow for visibility and could be sterilized using ethylene oxide (EO) gas. Scaffold insertion would be difficult, since access to the center can only be made from one end. Manufacture of this type of design would be difficult requiring adhesive when attaching the corrugated tubing to the center section. In addition, corrugated tubing is expensive and custom lengths and configurations would be needed. The limiting factors for this design are scaffold insertion, manufacturability and cost. Again, usability is a concern. A cylindrical shape would be challenging to work with and place in the incubator as it would roll and is not stackable. This design would be difficult to configure in regards to tubing adjustability, as the tubing would need to slide and adjust as the

corrugated tubing is pulled or pushed. The overall evaluation of Initial Design 3 is summarized below:

- User friendly design: No- chamber would be easy to assemble but scaffold insertion would be difficult and chamber would roll when on a flat surface
- Seal-ability: Easy- entire chamber would be closed and only need a leak-proof sealing cap
- Availability of materials and components: Custom- corrugated tubing would need to be custom ordered and designed
- Adjustability: High- chamber length can be easily adjusted to scaffold length
- Cost: High- due to corrugated tubing, adhesive, manufacturing and overall complex and custom design

Initial Design 4

Description: The fourth initial design idea consisted of a clear plastic rectangular box with snap-on sealing lid. The box and lid could be separate pieces or possibly held together by a hinge. The box would be completely open on top, allowing for complete access into the chamber, and the lid would contain a rubber o-ring. When snapped together the lid would create a leak-proof seal against the top of the box. Two centered holes would be made on opposite sides in the bottom of the box, and the tubing and scaffold would be positioned in the middle. During set-up, the scaffold and tubing would be placed in the center of the bottom section, prior to placement and closure of the lid.

Initial Design 4 is modeled in Figures 2.7 and 2.8 below.

Figure 2.7- Exploded 3D model of Initial Design 4. An exploded view shows the overall design concept with the top and bottom sections separated. The scaffold and tubing is placed in the center of the bottom section.

Figure 2.8- Assembled 3D model of Initial Design 4. An assembled view shows the overall design concept with the top and bottom sections snapped and sealed together. The scaffold and tubing is encompassed within the box.

Evaluation: The biggest advantage of this design is that the snapping lid would be easy to assemble, use and seal. This design would be easy to open, close and put together. The box design would be easy to configure in an incubator and is stackable. Scaffold placement would be simple, as it would be placed in the center of the bottom section. Clear plastic would allow for visibility and could be sterilized using ethylene oxide (EO) gas. Containers of this type are readily available in a number of shapes and sizes and are low in cost. No custom designed parts would be needed and manufacturing would be simple without the need for adhesive or any complex seal design. This design would be easy to build and materials are available. The scaffold length would be somewhat variable, but limited by the chamber length. The main drawback of this design is that the length of the chamber is not easily adjustable. The overall evaluation of Initial Design 4 is summarized below:

- User friendly design: Yes- chamber would be easy to assemble and scaffold would be easy to insert
- Seal-ability: Easy- only the lid would contain a sealing o-ring which would experience minimal wear
- Availability of materials and components: Off the Shelf- standard shaped container available in many sizes with no custom parts
- Adjustability: Limited- longest scaffold would be limited by chamber length
- Cost: Low- materials and components are off the shelf and readily available with nothing custom needed

Initial Design 5

Description: The final initial design idea consisted of a clear plastic cylindrical container with sealing snap-on lid. The cylindrical container would be open on top and the lid would contain a rubber o-ring seal. When snapped together the lid would create a leak-proof seal against the top of the cylindrical container. Two holes would be made in the lid. The tubing and scaffold would hang vertically from one of the holes, loop around in a u-shape, and exit through the second hole. Initial Design 5 is modeled in Figures 2.9 and 2.10 below.

Figure 2.9- Exploded 3D model of Initial Design 5. An exploded view shows the overall design concept with the top and bottom sections separated. The scaffold and tubing are looped into a u-shape and hang vertically from the lid.

Figure 2.10- Assembled 3D model of Initial Design 5. An assembled view shows the overall design concept with the top and bottom sections snapped and sealed together. The scaffold and tubing are encompassed within the cylindrical chamber.

Evaluation: The biggest advantage of this design is that the snapping lid would be easy to assemble, use and seal. Scaffold placement would be simple, as it would hang from the lid. Clear plastic would allow for visibility and could be sterilized using ethylene oxide (EO) gas. The chamber would be easy to configure in an incubator but is not stackable due to the tubing being attached to the lid. Containers of this type are readily available in a number of shapes and sizes and are low in cost. No custom designed parts would be needed and manufacturing would be simple without the need for adhesive or any complex seal design. This design would be easy to build and materials are available. The scaffold length would be somewhat variable, but limited by the container length. In addition, the tubing length would need to be adjusted each time the scaffold length is changed. The key difference and possible challenge in this design is

that the scaffold is oriented vertically. The overall evaluation of Initial Design 5 is summarized below:

- User friendly design: Yes- chamber would be easy to assemble and scaffold would be easy to insert
- Seal-ability: Easy- only the lid would contain a sealing o-ring which would experience minimal wear
- Availability of materials and components: Off the Shelf- standard shaped container available in many sizes with no custom parts
- Adjustability: Limited- longest scaffold would be limited by chamber length
- Cost: Low- materials and components are off the shelf and readily available with nothing custom needed

Evaluation of Initial Design Models and Concepts

A summary of the design criteria evaluated in the previous section is given in Table 2.1 below. Initial designs 1 through 5 were evaluated based on user friendly design, seal-ability, availability of materials and components, adjustability, and overall cost. The results of these evaluated criteria were used when deciding what initial designs to move forward with and build into physical prototypes.

	Initial Design 1	Initial Design 2	Initial Design 3	Initial Design 4	Initial Design 5
User Friendly Design	Yes	Somewhat	No	Yes	Yes
Seal-Ability	Difficult	Difficult	Easy	Easy	Easy
Availability of Materials and Components	Custom	Custom	Very Custom	Off the Shelf	Off the Shelf
Adjustability	Limited	High	High	Limited	Limited
Cost	Moderate to High	Moderate to High	High	Low	Low

Table 2.1- Evaluation of initial design ideas. The initial design ideas were evaluated based on the following criteria: user friendly design, seal-ability, availability of materials and components, adjustability, and overall cost.

Ultimately the final decision on what designs to move forward with and build into prototypes was based on seal-ability and availability of materials and components, along with consideration of the other above stated criteria. The highest priority was choosing a design that could be easily sealed and remain leak-proof, so that sterility could be maintained. Seal-ability is extremely important since a sterile environment is needed for cell and tissue growth (Fisher, Mikos and Bronzino 2007; Chen and Hu 2006). The next

consideration was choosing a design that could actually be built, utilizing materials and components that are readily available, since the aim of this thesis was to both design and build a BVM system for preclinical device testing. A design was needed that could be easily manufactured without the need for adhesive or custom parts. A more user friendly design was one of the primary needs of the BVM system and was the next consideration. Those designs in which scaffold placement was simple and overall usability was easy were given priority. Although cost was not a major deciding factor, the designs that utilized off the shelf components also had the advantage of being low cost. Finally, a more variable design with the ability to accommodate a wider range of stent sizes was the last consideration. In evaluating all the designs, adjustability became a minor consideration. Although a custom adjustable chamber would be nice, a longer scaffold could be easily accommodated in the other designs by simply choosing a larger container. Therefore, adjustability was given the least priority when choosing the designs with which to move forward and prototype. To summarize, the evaluated design criteria were given the following priorities:

1. Seal-ability
2. Availability of materials and components
3. User friendly design
4. Cost
5. Adjustability

After prioritizing the above criteria and evaluating the results found in Table 2.1, only initial designs 3, 4 and 5 met the top design criteria of being easy to seal. Additionally, initial designs 4 and 5 met the second criteria utilizing materials and components that are easily available and off the shelf, rather than needing to be custom designed or ordered. Early evaluation revealed initial designs 4 and 5 as the designs with the most potential, meeting the top 4 out of 5 design criteria stated above.

While searching for available materials and components, a simple solution was found to overcome the challenge of seal-ability and manufacturability which further narrowed the overall design considerations down to initial designs 4 and 5. By using a leak-proof plastic food container with snap-on sealable lid and built in o-ring, the basic chamber design was solidified and initial designs 4 and 5 were chosen to be prototyped. Initial designs 4 and 5 also had the advantage of being both user friendly and low cost designs. Figure 2.11 below shows commercially available plastic food containers with snap-on lids in several different shapes and sizes.

Figure 2.11- Leak-proof plastic food container with snap-on sealing lid.

A leak-proof plastic food container was chosen for the chamber design because it is easy to seal, made of clear biocompatible and sterilizable plastic, low cost and available in local stores. Leak-proof polypropylene food containers come in a variety of shapes, sizes and configurations with lids that have built-in silicone o-rings. The leak-proof food container meets the following design criteria stated in the introduction:

- ✓ An overall user friendly design so that assembly is simple and straightforward
 - Standard container configuration with snap-on lid
- ✓ Easy to seal leak-proof chamber for quick and sterile assembly
 - Lid has built-in silicone o-ring and latches that snap to seal
- ✓ Sterilizable by ethylene oxide (EO) gas
 - Polypropylene and silicone can withstand EO sterilization
- ✓ Optically clear chamber for easy visibility of scaffold, media and stent placement
 - All sides of container are see-through
- ✓ Easy scale-up so that multiple chambers can be configured in the incubator
 - Containers are stackable and modular
- ✓ Low cost
 - Approximately \$3-10 per container, depending on size
- ✓ Made of materials and components that are readily available and easy to obtain
 - Containers can be purchased in local stores or online
- ✓ Made of biocompatible materials so that cells are able to grow and function
 - Polypropylene and silicone are biocompatible materials

Design Prototypes

After identifying containers and identifying initial designs 4 and 5 as the best potential candidates, the next step was to build physical prototypes to evaluate and further narrow the selection to one final design. The following two prototypes were constructed by purchasing leak-proof plastic food containers from Linens-N-Things, fittings and connections from Value Plastics, Qosina, and McMaster-Carr and flexible tubing from Cole-Parmer. The two prototypes were assembled based on the above models of initial designs 4 and 5 and are shown and described below. Details on how the fittings were secured, specific parts used and the entire assembly process will be addressed in the following chapter after the final design has been chosen. The goal of this section was to build physical prototypes that could be held, compared, and evaluated by potential users in order to determine the final design.

Design Prototype 1

Description: The first prototype built was based on the model of initial design 4. A rectangular, clear, plastic, leak-proof food container with snap-on sealing lid was used for the chamber. The container used measured approximately 5" long x 3.5" wide x 2" deep. The lid contained a silicone o-ring seal and four snapping latches that locked to the bottom section securing the bottom and lid together. Two holes were made on opposite sides of the container and threaded luer lock to barbed connection fittings were secured within the holes. The scaffold was horizontally positioned between the fittings using the appropriate connections. Adjustment to scaffold length could be made by adding or

removing fittings. Photographs of the physical prototype built are shown below in Figures 2.12 and 2.13.

Figure 2.12- Side view of Prototype 1. A rectangular plastic leak-proof container with snap-on sealing lid was used for the chamber.

Figure 2.13- Top view of Prototype 1. The scaffold was horizontally positioned within the chamber using the appropriate connections.

Design Prototype 2

Description: The second prototype built was based on the model of initial design 5. A tall rectangular, clear, plastic, leak-proof food container with snap-on sealing lid was used. The container used measured approximately 4" wide x 4" wide x 4.5" tall. The lid contained a silicone o-ring seal and four snapping latches that locked to the bottom section securing the bottom and lid together. Two holes were made in the lid of the container and threaded luer lock to barbed connection fittings were secured within the holes. The scaffold was hung vertically from the lid using the appropriate connections and the tubing was configured in a u-shape. Adjustment to scaffold length could be made by changing the opposite side tubing length. A photograph of the physical prototype built is shown below in Figure 2.14.

Figure 2.14- Front view of Prototype 2. A rectangular plastic leak-proof container with snap-on sealing lid was used for the chamber. The scaffold hung vertically from the lid.

Evaluation of Design Prototypes

Prototypes 1 and 2 were evaluated in comparison with the original BVM system and to each other based on an expanded list of key criteria. The original BVM system along with physical Prototypes 1 and 2 were handled and used by several individuals in order to evaluate overall usability and ease of scaffold insertion. Measurable criteria evaluated included volume, maximum scaffold length and diameter, and maximum stent size. Observable criteria included graft orientation, closure mechanism, need for assembly tools, and stack-ability. Other criteria included cost and chamber design. Table 2.2 below summarizes the results of the compared criteria of the original BVM system, Prototype 1 and Prototype 2.

	Original BVM System	Design Prototype 1	Design Prototype 2
Overall Usability	moderate	easy	easy
Scaffold Insertion	difficult	easy	moderate
Chamber Volume	25 ml	350 ml	700 ml
Graft Orientation	horizontal	horizontal	vertical
Closure Mechanism	screws	locking snaps	locking snaps
Assembly tools required	yes	no	no
Stackable	no	yes	no
Max Scaffold Length	4.5 cm	5.5 cm	6 cm
Scaffold Diameters	3-4 mm	2-6 mm	2-6 mm
Maximum Stent Length	25 mm	35 mm	40 mm
Stent Diameters	3-4 mm	2-6 mm	2-6 mm
Cost	moderate	low	low
Chamber Design	custom	off the shelf	off the shelf

Table 2.2- Evaluation of design prototypes versus original BVM system. The design prototypes were compared to the original BVM system and evaluated based on objective, measurable and observable criteria.

Prototype 1 and Prototype 2 met all design criteria stated in the introduction. When compared to the original BVM system, both prototypes also had the advantage of being more user friendly with easier scaffold placement and utilized a simpler closure mechanism without the need for assembly tools. The overall usability and ease of scaffold insertion was evaluated by the individuals that make up the 2008 BVM Lab Group, after handling and using all three designs. It was observed that locking snaps provided a quick and simple sealing method when compared with the previous method of tightening screws. Also, by using rotating fittings in the prototype designs the need to bend, push or twist the scaffold during insertion was eliminated.

In addition to usability characteristics, the prototypes were lower cost and chamber designs were non-custom and off the shelf. Maximum scaffold length and diameter was also greater in the prototype designs, resulting in the accommodation of a wider range of stent sizes. The stent sizes that could be inserted into the scaffold were estimated by taking the maximum scaffold length and subtracting 2 cm to take into account the amount of material used for attachment and the amount of non-useable area.

As described above, Prototypes 1 and 2 have many advantages over the original BVM system. The next step in designing a new, more ideal BVM was to compare Prototypes 1 and 2 to each other. The significant difference in design between Prototype 1 and Prototype 2 was scaffold orientation. The original BVM system utilizes a horizontal scaffold orientation and previous evaluation and use of a vertically oriented scaffold have revealed difficulties in cell sodding, with the cells tending to migrate to the bottom of the scaffold due to gravity (KO Cardinal, unpublished data). Therefore the

biggest disadvantage of Prototype 2 was the vertical scaffold orientation. Chamber volume was also compared and considered. Although not critical, a smaller volume is more advantageous since it requires less media to fill. Both prototypes were much larger in volume than the original BVM system, however, Prototype 2 was twice the volume of Prototype 1. In addition to a horizontal scaffold orientation and smaller chamber volume, Prototype 1 also has the advantage of being stackable when compared to Prototype 2. As a result of these advantages, Prototype 1 was chosen to be used for the final design.

Final Design

Based upon the previously discussed evaluation, Prototype 1 was chosen to be used as the final design. The following chapter will review in detail the design, construction and set-up of this Ideal BVM system as well as the testing performed to validate and verify its functionality and use.

3. FINAL DESIGN OF IDEAL BVM

Introduction

As stated in the previous chapter, design and construction of bioreactors are important in tissue engineering research because they directly provide the *in vitro* environment for tissue growth (Chen and Hu 2006). A bioreactor must operate over sustained periods of time under aseptic conditions so that cells are able to grow and function. The goal is to create a system that reliably and reproducibly supports the growth of functional living tissue (Martin, Wendt and Heberer 2004; Ratcliffe and Niklason 2002). In the case of a Blood Vessel Mimic (BVM), the goal is to create an environment to cultivate tissue engineered blood vessels (TEBV) in order to serve as a living vessel model for preclinical device testing (Cardinal, et al. 2006). The aim of the work in the previous chapter was to improve upon the original BVM system by developing a new, more ideal design. A simpler, easier to use and more variable design was selected from several potential ideas and an initial physical prototype was built and modeled.

The new Ideal BVM system developed and prototyped in the previous chapter consists of an off the shelf leak-proof plastic horizontal rectangular vessel chamber with a luminal inlet, luminal outlet and extra luminal outlet. The outlets lead to a two-port media reservoir, with stopcocks controlling the flow path. The scaffold is attached to two rotating luer fittings which are attached to the luminal inlet and luminal outlet ports located within the vessel chamber. Adjustments to length are made by adding or removing luer fittings. All sides of the chamber are optically clear allowing for easy visualization of the scaffold, media and stent placement. The chamber itself is sealed by a

built-in silicone o-ring and clamped together by closing four snapping latches. The system can be sterilized using ethylene oxide (EO) gas. The specific design criteria met by the Ideal BVM were summarized in Chapter 2 and provided the motivation for moving forward with this design. Therefore, the aim of the work in this chapter is to describe in detail the finalized design, construction and set-up of this Ideal BVM system and the testing performed to validate and verify its functionality and use.

Materials and Methods

Ideal BVM System

This section outlines the steps taken to build the new Ideal BVM system. The assembly process is described in detail and includes the specific parts used along with detailed illustrations. While this section provides the materials and methods for construction of the Ideal BVM system, a detailed list of parts and drawings, as well as complete step-by-step directions can also be found in Appendix B and C respectively. The new, Ideal BVM system was constructed as follows.

To begin, the luminal inlet, luminal outlet and extra luminal outlet were created and assembled. Three 15/64" holes were drilled into the sides of a 350 ml, 5" x 4" x 2", semitransparent, polypropylene rectangular snap and seal leak-proof food container (Lock & Lock P/N HPL806). Two holes were drilled on opposite sides, one in the center of each face, directly across from one another. The centered hole on the left hand side became the luminal inlet and the centered hole on the right hand side became the luminal outlet. A third hole was drilled adjacent to the centered face hole on the right hand side of the container. This hole became the extra luminal outlet. Using a razor blade, the edges of the holes were cleaned of any plastic debris that resulted from the use of the drill. A soft black Buna-N o-ring, AS568A dash number 006, durometer 50A, 1/4" OD, 1/8" ID, 1/16" W (McMaster-Carr P/N 2418T112) was slid onto a white nylon, female luer lug style panel mount connector, 1/4-28 UNF to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLB230-1) and threaded through one of the drilled holes with the barbed portion exiting the container. A second o-ring was placed over the barbed section and a

white nylon, 1/4-28 UNF panel mount lock nut (Value Plastics P/N LNS-1) was screwed on tightly, pressing the o-rings against the inside and outside of the container walls, creating a leak-proof seal. Once securely tightened, a drop of Loctite 4011 medical grade adhesive (McMaster-Carr P/N 1818A4) was placed between the lock nut and the threaded portion of the barbed fitting to secure it in place and prevent loosening. This procedure was repeated for the remaining drilled holes. Figures 3.1 and 3.2 below illustrate this assembly procedure.

Figure 3.1- Assembly of the luminal inlet and luminal outlets. Each of the three 15/64" holes was assembled using two o-rings, a female luer lug panel mount and a lock nut.

Figure 3.2- Completed assembly of the luminal inlet and luminal outlets. The female luer lug panel mounts were threaded through the holes drilled in the plastic container and the lock nuts were tightened, pressing the o-rings against the container walls creating a seal.

To complete the luminal inlet assembly a 2" piece of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) was inserted onto the barbed fitting of the luminal inlet exiting the left side of the container. A white nylon, female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1) was attached to the open end of the silicone tubing attached to the luminal inlet. Finally, a three-way polycarbonate stopcock with two female and one male luer lock ends (Value Plastics P/N VPB1000079N) was attached to the female luer end on the luminal inlet tubing. The assembly process described is illustrated in Figures 3.3 and 3.4 below.

Figure 3.3- Luminal inlet tubing assembly. The luminal inlet consists of a short piece of silicone tubing, a female luer thread barbed fitting, and a three-way stopcock.

Figure 3.4- Completed luminal inlet tubing assembly.

To complete the luminal outlet and extra luminal outlet assembly a 1" piece of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) was inserted onto the barbed fitting of the luminal outlet and a 5" piece of the same silicone tubing was inserted onto the barbed fitting of the extra luminal outlet, both exiting the right side of the container. A white nylon, male luer integral lock ring to 200 series barb, 1/8" ID tubing (Value Plastics P/N MTLL230-1) was attached to the open end of the silicone tubing attached to the luminal outlet. A one-way polycarbonate stopcock with one female and one male luer lock end (Value Plastics P/N VP455980) was attached to the male luer end on the luminal outlet tubing. A white nylon, female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1) was attached to the male end of the one-way stopcock, and a 2" piece of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) was inserted onto the barbed fitting of the female luer attached to the one-way stopcock.

Next, the open ends of the silicone tubing attached to the luminal outlet and extra luminal outlet were attached to a white nylon, Y-connector with 200 series barbs, 1/8" ID tubing (Value Plastics P/N Y230-1). The attachment of the luminal outlet and extra luminal outlet tubing to the Y-connector created one main outlet. Finally, a 1" piece of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) was inserted onto the barbed fitting of the Y-connector and a white nylon, male luer integral lock ring to 200 series barb, 1/8" ID tubing (Value Plastics P/N MTLL230-1) was inserted into the open end of the silicone tubing attached to the Y-connector. A one-way polycarbonate stopcock with one female and one male luer lock end (Value Plastics P/N VP455980) was attached to the male luer end on the Y-connector tubing. Figure 3.5

provides an overall view of the assembly process, and Figures 3.6 and 3.7 provide a closer view of the luminal outlet and extra luminal outlet assembly.

Figure 3.5- Overall view of luminal outlet and extra luminal outlet assembly.

Figure 3.6- Detailed view of luminal outlet and extra luminal outlet assembly. The luminal outlet assembly consists of a one-way stopcock, while the extra luminal outlet is a simple tube. The luminal outlet and extra luminal outlet are both attached to a Y-connector and meet to form one outlet ending with a one-way stopcock.

Figure 3.7- Completed luminal outlet and extra luminal outlet assembly. The two outlets meet at the Y-connector combining to form one main outlet.

To complete the inside of the chamber, two male-to-male luer lock connectors, with one rotating collar (Qosina P/N 17644), were attached to the female luer lock connector ends of the luminal inlet and luminal outlet, extending into the chamber. The male-to-male connectors were attached so that the rotating collars extended into the chamber and were free to rotate. Figures 3.8 and 3.9 below illustrate this assembly procedure. To shorten the distance between the two ends of the male-to-male luer lock connectors, white nylon male luer integral lock ring to female luer thread style couplers (Value Plastics P/N LC34-1) can be attached prior to the attachment of the male-to-male luer lock connectors. The attachment of one coupler to both the luminal inlet and luminal outlet is illustrated in Figures 3.10 and 3.11 below and will be detailed in a later section.

Figure 3.8- Attachment of male-to-male luer lock connectors. Male-to-male luer lock connectors, with one rotating collar, were attached to the female luer lock connector end of the luminal inlet and luminal outlet.

Figure 3.9- Completed attachment of male-to-male luer lock connectors. Male-to-male luer lock connectors were attached so that the end extending into the chamber remained free to rotate.

Figure 3.10- Addition of male-to-female luer lock couplers. Prior to the attachment of the male-to-male luer lock connectors, male-to-female couplers can be added to shorten the distance between the rotating ends.

Figure 3.11- Attachment of male-to-female luer lock couplers. Male-to-male luer lock connectors were attached so that the end extending into the chamber remained free to rotate.

The final complete new Ideal BVM system, prior to scaffold insertion, is diagramed below in Figure 3.12. The lid is attached by closing four snapping latches and a built-in o-ring creates a leak-proof seal. The Ideal BVM system was part of the overall bioreactor configuration, which will be diagramed in a later section.

Figure 3.12- Final complete new Ideal BVM system.

Two-Port Media Reservoir

This section outlines the steps taken to build the two-port media reservoir for the new Ideal BVM system. After construction of the luminal inlet and luminal outlets of the Ideal BVM system it was observed that this configuration would also improve the function and use of the inlet and outlet ports on the media reservoir lid, allowing reservoir construction to be accomplished without the need for a large amount of adhesive. Therefore, the same basic construction used to build the Ideal BVM system luminal inlet and luminal outlets was applied to the two-port media reservoir. The assembly process is described in detail and includes the specific parts used along with detailed illustrations. While this section provides the materials and methods for construction of the two-port media reservoir, a detailed list of parts and drawings, as well as complete step-by-step directions can also be found in Appendix B and D respectively.

The two-port media reservoir for the Ideal BVM system was created as follows. Two 15/64" holes were drilled into the lid of a BD Falcon™ 50 ml conical-bottom polypropylene tube (BD Biosciences P/N 352098). Using a razor blade, the edges of the holes were cleaned of any plastic debris that resulted from the use of the drill. Using the same technique described previously, a soft black Buna-N o-ring, AS568A dash number 006, durometer 50A, 1/4" OD, 1/8" ID, 1/16" W (McMaster-Carr P/N 2418T112) was slid onto a white nylon, female luer lug style panel mount connector, 1/4-28 UNF to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLB230-1) and threaded through one of the drilled holes with the barbed portion exiting the top of the lid. A second o-ring was placed over the barbed section and a white nylon, 1/4-28 UNF panel mount lock nut

(Value Plastics P/N LNS-1) was screwed on tightly, pressing the o-rings against the top and bottom of the lid, creating a leak-proof seal. Once securely tightened, a drop of Loctite 4011 medical grade adhesive (McMaster-Carr P/N 1818A4) was placed between the lock nut and the threaded portion of the barbed fitting to secure it in place and prevent loosening. This procedure was repeated for the second drilled hole. Next, a 1" piece of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) was attached to a white nylon, male luer integral lock ring to 200 series barb, 1/8" ID tubing (Value Plastics P/N MTLL230-1) and secured to one of the female luer fittings extending from the bottom of the lid. This port, with the attached tubing, became the outlet. The assembly process described is illustrated in Figures 3.13 and 3.14 below.

Figure 3.13- Assembly of two-port media reservoir lid. Each of the two 15/64" holes was assembled using two o-rings, a female luer lug panel mount and a lock nut. A short piece of tubing was inserted onto a male luer lock barbed fitting and attached to the assembly.

Figure 3.14- Assembled two-port media reservoir. The female luer lug panel mounts were threaded through the drilled holes and the lock nuts were tightened, pressing the o-rings against the top and bottom of the lid, creating a seal. A short piece of silicone tubing was attached and became the outlet.

To complete the two-port media reservoir two 10" pieces of Tygon® silicone tubing, 1/8" ID x 1/4" OD (Cole-Parmer P/N WU-95702-06) were inserted onto the barbed fittings of the inlet and outlet exiting the media reservoir lid. A white nylon, male luer integral lock ring to 200 series barb, 1/8" ID tubing (Value Plastics P/N MTLL230-1) was attached to the open end of the silicone tubing attached to the outlet. Likewise, a white nylon, female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1) was attached to the open end of the silicone tubing attached to the inlet. Figures 3.15 and 3.16 below illustrate this assembly procedure. The two-port media reservoir was part of the overall bioreactor configuration, which will be diagrammed in a later section.

Figure 3.15- Inlet and outlet tubing assembly of two-port media reservoir. A male luer lock barbed fitting is attached to the outlet and a female luer thread barbed fitting is attached to the inlet.

Figure 3.16- Final completed assembly of two-port media reservoir.

Scaffold Setup and Insertion

The scaffold for the new Ideal BVM system was setup as follows. Expanded polytetrafluoroethylene (ePTFE), 3 mm and 4 mm inner diameter (Impra Bard, Inc., Tempe, AZ), was used as the scaffold material. Two white nylon, female luer thread style to 200 series barb fittings were inserted into the open ends of the ePTFE scaffold. White nylon, female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1) were used for the 4 mm inner diameter scaffolds and white nylon, female luer thread style to 200 series barb, 3/32" ID tubing (Value Plastics P/N FTLL220-1) were used for the 3 mm inner diameter scaffolds. The ends of the scaffold were sutured onto the barbed ends of the female luer thread fittings using 2.0 SofsilksTM suture material. Figures 3.17 and 3.18 below illustrate this procedure. Depending on the number of male luer integral lock ring to female luer thread style couplers used between male-to-male rotating fittings, scaffolds were cut into either 5.5 cm, 4.5 cm or 3.5 cm lengths, corresponding to 0, 1, or 2 couplers respectively. See Table 3.1 below or Appendix E for a summary of scaffold length versus number of couplers used.

Figure 3.17- Scaffold assembly. Two female luer thread barbed fittings were inserted into the open ends of the ePTFE scaffold.

Figure 3.18- Completed scaffold assembly. The ends of the ePTFE scaffold were sutured to the two female luer thread barbed fittings.

Illustration	# of Couplers	Scaffold Length
0	5.5 cm
1	4.5 cm
2	3.5 cm

Table 3.1- Scaffold length versus number of couplers used.

To secure the scaffold inside the Ideal BVM system, the female luer thread fittings attached to the scaffold were inserted into the male-to-male luer lock fittings attached to the luminal inlet and luminal outlet. The rotating collars of the male-to-male luer lock fittings were tightened around the female luer thread fittings attached to the scaffold. The inserted scaffold is illustrated below in Figure 3.19.

Figure 3.19- Scaffold insertion. The scaffold is secured inside the BVM system by tightening the rotating collars of the male-to-male luer lock fittings around the female luer thread fittings attached to the scaffold.

Bioreactor Configuration

The complete bioreactor configuration consisted of the Ideal BVM system and two-port media reservoir connected in series. To construct the bioreactor system the male luer lock fitting attached to the two-port media reservoir outlet tubing was secured to the female luer thread fitting of the three-way stopcock attached to the luminal inlet of the Ideal BVM system. Next, the female luer thread fitting attached to the two-port media

reservoir inlet tubing was secured to the male luer lock fitting of the one-way stopcock attached to the main luminal outlet of the Ideal BVM system. This completed the Ideal BVM system bioreactor assembly. The Ideal BVM bioreactor system is illustrated in Figure 3.20 below.

Figure 3.20- Ideal BVM system bioreactor configuration.

Cost Analysis

Due to the goal of creating a cost-effective, high-throughput testing environment, the cost of each individual component and part used to create the Ideal BVM system and two-port media reservoir was determined. The cost of all components and parts were summed and the total cost per individual completed Ideal BVM system and two-port media reservoir was calculated.

Functional Verification

To verify that the Ideal BVM system and two-port media reservoir functioned properly without leakage, a complete bioreactor configuration was setup and used. A lab member completed a full sterile Ideal BVM setup including scaffold assembly and insertion, media injection, and connection of the Ideal BVM and two-port media reservoir in series. Flow through the bioreactor system was initiated by placing a section of the two-port media outlet tubing through a peristaltic pump. The Ideal BVM functional verification setup is illustrated in Figure 3.21 below.

Figure 3.21- Ideal BVM functional verification setup. Flow was initiated by placing a section of the two-port media outlet tubing through a peristaltic pump.

Comparison of Ideal BVM System to Original BVM System

To compare the Ideal BVM system and the original BVM system a lab member completed a full sterile side-by-side setup of both systems. Setup included scaffold assembly and insertion, media injection, and connection of the BVM systems and two-port media reservoirs in series. Flow through the bioreactor systems was initiated by placing a section of the two-port media outlet tubing through a peristaltic pump. The Ideal BVM system and original BVM system were compared and user feedback was gathered on overall usability, ease of scaffold insertion, user interface features, connections and fittings, bioreactor assembly, seal-ability, aseptic use and setup, and general component configuration. The user feedback survey can be found in Appendix F.

Blood Vessel Development

The Ideal BVM system was tested for its ability to cultivate an *in vitro* blood vessel mimic. Following scaffold insertion and priming of the system with media, human umbilical artery smooth muscle cells (HUASMC) were pressure seeded onto the luminal surface of the ePTFE scaffold. The BVM system was placed in an incubator at 37 °C, 5% CO₂, and connected to a peristaltic pump. The pump generated flow through the lumen of the vessel over a time period of one day. After one day, the vessel was removed from the chamber and fixed in Histochoice for evaluation. Cell deposition and cell adherence to the vessel were evaluated using a scanning electron microscope. The fixed vessel was cut in half longitudinally and images of the luminal surface were acquired to

determine if the Ideal BVM system was capable of successfully supporting cell sodding and cultivation.

Results

Ideal BVM System

The Ideal BVM System was easy to assemble and build. All components were off the shelf and readily available, resulting in a design that was repeatable and consistent. No special tooling was needed and assembly was simple and straightforward. The leak-proof snap and seal container was easy to work with and durable. Holes were drilled cleanly with a handheld power drill and an ordinary 15/64" drill bit. By threading the female luer lug panel mounts directly into the plastic the need for a large amount of adhesive was eliminated. The double o-ring design created a leak-proof seal and allowed for a clean and simple overall design. Tubing was easily attached to the barbed fittings exiting the chamber and female luer threads allowed for easy attachment of male-to-male rotating luer lock fittings. Male luer lock and female luer thread connectors and barbed fittings made tubing and stopcock assembly easy. Due to the simplicity of design, availability of components and easy assembly, it was possible to construct a large number of identical systems. Therefore, thirty Ideal BVM systems were constructed for immediate use. Assembled Ideal BVM systems are shown below in Figures 3.22 and 3.23.

Figure 3.22- Ideal BVM System.

Figure 3.23- Assembled Ideal BVM systems. Tubing was attached to the barbed fittings exiting the sides of the container (a) and male-to-male rotating fittings were attached to the female luer thread fittings extending into the chamber (b) and (c). The sealing double o-ring design (d) eliminated the need for a large amount of adhesive (e).

Two-Port Media Reservoir

Two-port media reservoir lids were also easy to assemble and build. The sealing double o-ring design eliminated the need for a large amount of adhesive and allowed for a clean and simple overall design, while providing a functional seal. Tubing was easily attached to the barbed fittings exiting the top of the lid and the 50 ml tube was easy to screw onto the lid. The female luer thread fittings exiting the bottom of the lid also made it easy to attach a short piece of tubing to create the outlet. Due to the simplicity of design, availability of components and easy assembly, thirty two-port media reservoirs were constructed for immediate use. Assembled two-port media reservoirs are shown below in Figures 3.24 and 3.25.

Figure 3.24- Two-port media reservoir.

Figure 3.25- Assembled two-port media reservoirs. The sealing double o-ring design (a) eliminated the need for a large amount of adhesive (b). Tubing was attached to the barbed fittings exiting the top of the lid (c) and a short piece of tubing was secured to the female luer thread fitting exiting the bottom (d). Thirty two-port media reservoirs were constructed for immediate use (e).

Scaffold Setup and Insertion

Scaffold setup was simple and straightforward. The female luer thread barbed fittings were easy to insert into the ePTFE scaffold and the Sofsilks™ suture material was easy to tie and successfully secured the scaffold in place. The female luer thread barbed fittings were also easy to insert into the male-to-male rotating fittings within the Ideal

BVM chamber. The rotating collars of the male-to-male luer lock fittings allowed insertion to be performed without bending or twisting of the scaffold material. The female luer thread end attached to the scaffold could be held in place while the male luer lock rotating collar was tightened around it. These features also permitted fast and aseptic insertion of the scaffold within the Ideal BVM chamber. Scaffold setup and insertion are shown below in Figures 3.26 and 3.27.

Figure 3.26- Scaffold assembly.

Figure 3.27- Scaffold insertion into Ideal BVM system.

An advantage of this new scaffold setup was that scaffold length and diameter were easily adjustable and variations were easily accommodated within the system. By choosing the correct size of female luer thread barbed fittings a number of diameters of ePTFE scaffold could be used. In addition, by using Table 3.1, scaffold length was easily adjustable by varying the number of couplers used in the inner BVM chamber assembly. The ability to vary scaffold length and diameter allows for the accommodation a large number of stents sizes to be tested within the Ideal BVM system.

Bioreactor Configuration

The complete bioreactor configuration, consisting of the Ideal BVM system and two-port media reservoir connected in series, was easy to setup with the need for only two simple connections. Specific placement of male luer lock and female luer thread fittings ensured proper setup and orientation every time. Only one configuration existed for setting up the bioreactor system, since the fittings of the Ideal BVM system and two-port media reservoir were designed as male-to-female connections. This new design eliminated the confusion and complications faced in determining the correct inlet and outlet orientation when connecting the two-port media reservoir to the Ideal BVM system. The complete Ideal BVM bioreactor configuration is shown below in Figure 3.28.

Figure 3.28- Complete bioreactor configuration. The Ideal BVM system and two-port media reservoir were connected in series. Specific placement of male luer lock and female luer thread fittings ensured proper setup and orientation every time.

Cost Analysis

The cost of each individual component and part used to create the Ideal BVM system and two-port media reservoir and the total cost per individual completed Ideal BVM system and two-port media reservoir is summarized in Tables 3.2 and 3.3 below. The total cost per individual completed Ideal BVM system was \$9.71 and the total cost per individual completed two-port media reservoir was \$5.54. The total cost of the completed bioreactor configuration, including both the Ideal BVM system and two-port media reservoir was \$15.25.

Component	Company	Part Number	Qty per BVM	Cost per Unit	Total
Leak-Proof Chamber	Lock & Lock	HPL806	1 EA	3.00	3.00
Buna-N O-Ring	McMaster-Carr	2418T112	6 EA	0.07	0.42
Loctite 4011 Adhesive	McMaster-Carr	1818A4	3 drops	0.02	0.06
Female Luer Lug Panel Mount-1/8" Barb	Value Plastics	FTLB230-1	3 EA	0.17	0.51
Lock Nut	Value Plastics	LNS-1	3 EA	0.08	0.24
Male Luer Lock-1/8" Barb	Value Plastics	MTLL230-1	2 EA	0.14	0.28
Female Luer Thread-1/8" Barb	Value Plastics	FTLL230-1	2 EA	0.12	0.24
Y-Connector-1/8" Barbs	Value Plastics	Y230-1	1 EA	0.22	0.22
Male-Female Coupler	Value Plastics	LC34-1	2 EA	0.16	0.32
1-Way Stopcock	Value Plastics	VP455980	2 EA	0.64	1.28
3-Way Stopcock	Value Plastics	VPB1000079N	1 EA	0.64	0.64
Male-to-Male Rotating Luer Lock	Qosina	17664	2 EA	0.26	0.52
1/8" ID Silicone Tubing	Cole-Parmer	WU-95702-06	11 inches	0.18	1.98
					\$9.71

Table 3.2- Total cost per individual completed Ideal BVM system.

Component	Company	Part Number	Qty per Reservoir	Cost per Unit	Total
BD Falcon™ 50 ml Tube	BD Biosciences	352098	1 EA	0.54	0.54
Buna-N O-Ring	McMaster-Carr	2418T112	4 EA	0.07	0.28
Loctite 4011 Adhesive	McMaster-Carr	1818A4	2 drops	0.02	0.04
Female Luer Lug Panel Mount-1/8" Barb	Value Plastics	FTLB230-1	2 EA	0.17	0.34
Lock Nut	Value Plastics	LNS-1	2 EA	0.08	0.16
Male Luer Lock-1/8" Barb	Value Plastics	MTLL230-1	2 EA	0.14	0.28
Female Luer Thread-1/8" Barb	Value Plastics	FTLL230-1	1 EA	0.12	0.12
1/8" ID Silicone Tubing	Cole-Parmer	WU-95702-06	21 inches	0.18	3.78
					\$5.54

Table 3.3- Total cost per individual completed two-port media reservoir.

Functional Verification

The Ideal BVM system was used in conjunction with a two-port media reservoir and functioned properly without any leakage. The scaffold was inserted aseptically and the lid was easy to attach and created a leak-proof seal. The system was injected with media which was pumped through the system via a peristaltic pump. Flow was created through the luminal inlet and exited the luminal outlet and extra luminal outlet. The Ideal BVM systems were EO sterilized prior to use. Photos of the Ideal BVM system being setup and being attached to the peristaltic pump are shown below in Figures 3.29 and 3.30.

Figure 3.29- Setup of the Ideal BVM bioreactor system. The scaffold was inserted in an aseptic manner (a) and the two-port media reservoir and Ideal BVM were connected in series (b).

Figure 3.30- Attachment of the Ideal BVM bioreactor system to a peristaltic pump. The bioreactor system functioned properly without leakage.

The luminal inlet, luminal outlet and extra luminal outlet configuration permitted variation in flow path. Transluminal and transmural flow were easily controlled via the one-way stopcock attached to the luminal outlet. By closing off the luminal outlet path, through the use of the one-way stopcock, transmural flow through the porous ePTFE scaffold wall was created, with flow exiting the extra luminal outlet. Transmural flow was used for conditioning and cell seeding of the scaffold material. By opening the one-way stopcock, transluminal flow was restored through the luminal outlet. The one-way stopcock was easy to use and provided a quick and simple method for controlling the flow path. The lever was easy to turn and once in position flow was immediately and firmly blocked. The merging of the luminal outlet and extra luminal outlet into one main

outlet via the Y-connector made attachment to the two-port media reservoir simple with the need for only one connection. The three-way stopcock attached to the luminal inlet easily functioned as both a flow path and cell sodding port. With two female luer thread fittings, the three-way stopcock allowed the Ideal BVM system to be connected to the two-port media reservoir as well as maintain an extra port for cell sodding and media injection.

Two-port media reservoirs were used in conjunction with the Ideal BVM system and also functioned properly without any leakage. Liquid was pulled from the media reservoir through the short outlet tube extending into the media and pumped through the bioreactor system via a peristaltic pump. Liquid drops could be seen falling from the inlet port, allowing for quick and easy visualization of flow as well as removal of air bubbles. Media reservoirs were EO sterilized prior to use. A photo of the media reservoir setup is shown below in Figure 3.31.

Figure 3.31- Two-port media reservoirs in use. The two-port media reservoirs functioned properly without leakage. Flow was established via a peristaltic pump.

Comparison of Ideal BVM System to Original BVM System

The Ideal BVM system and original BVM system were setup side-by-side by a lab member and a user feedback survey was completed which compared the two systems. Figures 3.32 and 3.33 below show the two systems being setup side-by-side and in the incubator connected to a peristaltic pump. The criteria evaluated and the results of the user feedback survey are summarized in Table 3.4. The completed user feedback survey can be found in Appendix F.

Figure 3.32- Side-by-side setup of original BVM and Ideal BVM systems. A lab member performed a sterile setup of both systems.

Figure 3.33- Side-by-side setup of original BVM and Ideal BVM bioreactor systems. Both systems were setup in the incubator and connected to a peristaltic pump to create flow.

	Original BVM System					Ideal BVM System				
	Difficult		Easy			Difficult		Easy		
Overall Usability	1	2	3	4	5	1	2	3	4	5
Setup	1	2	3	4	5	1	2	3	4	5
Scaffold Assembly	1	2	3	4	5	1	2	3	4	5
Scaffold Insertion	1	2	3	4	5	1	2	3	4	5
Media Injection	1	2	3	4	5	1	2	3	4	5
Seal-Ability	1	2	3	4	5	1	2	3	4	5
Aseptic Use	1	2	3	4	5	1	2	3	4	5
Bioreactor Assembly	1	2	3	4	5	1	2	3	4	5
Control of Flow	1	2	3	4	5	1	2	3	4	5
Adjustability	1	2	3	4	5	1	2	3	4	5
	Awkward		Convenient			Awkward		Convenient		
Components and Fittings	1	2	3	4	5	1	2	3	4	5
	Poor		Excellent			Poor		Excellent		
Component Configuration	1	2	3	4	5	1	2	3	4	5

Table 3.4- Summary of user feedback survey. The Ideal BVM system and original BVM system were compared based on the criteria listed.

Based on the user feedback survey comments one of the advantages of the Ideal BVM system was that the chamber lid can be sterilized separately from the rest of the BVM chamber. In addition, the lid is not permanently attached to BVM chamber, and does not need to be held open, freeing up a second hand for use during scaffold insertion and work in the hood. Both these features were helpful in maintaining sterility and made setup easier. Another noted advantage was that the four snapping latches of the lid eliminated the need for tools when closing and sealing the BVM chamber. The Ideal BVM system also utilized stopcocks instead of side clamps to control flow and since the BVM chamber is not filled to the top with media the need to eliminate air bubbles during setup was no longer a challenge. Finally, with inlet and outlet fittings arranged so that only one configuration exists to setup the bioreactor system time was not wasted and there was no confusion when connecting the Ideal BVM system to the two-port media reservoir.

Based on the user feedback survey comments one of the disadvantages of the Ideal BVM system was that a larger volume of media was needed to fill the BVM chamber. Also, the larger container takes up more space in the incubator. However, it is important to note that the increased chamber volume only requires a one-time fill, as opposed to the reservoirs which are replaced every 3 days, and that these larger containers are stackable. The disadvantages, therefore, are offset by the advantages of the Ideal BVM system in comparison to the original BVM system.

Blood Vessel Development

The Ideal BVM system successfully cultivated an *in vitro* blood vessel mimic by supporting cell sodding and cultivation. The scaffold was aseptically inserted and human umbilical artery smooth muscle cells (HUASMC) were pressure sodded onto the luminal surface of the ePTFE scaffold. Cells adhered to the scaffold surface and were distributed along the length of the vessel. Scanning electron microscope (SEM) images of an ePTFE scaffold surface are shown below in Figures 3.34 and 3.35. An SEM image of bare ePTFE, which represents the scaffold prior to cell sodding, is shown for reference followed by SEM images of the ePTFE with cells adhered to the surface.

Figure 3.34- SEM image of bare ePTFE scaffold surface.

Figure 3.35- SEM images of ePTFE scaffold surface with cells. The Ideal BVM system successfully cultivated an *in vitro* blood vessel mimic with cell deposition and cell adherence to the scaffold surface.

Discussion and Conclusions

The new Ideal BVM system and two-port media reservoirs were successfully built and implemented. The work described in this chapter supports the use of the Ideal BVM system for cultivation of *in vitro* blood vessel mimics. The finalized design and setup procedures were described in detail with testing performed to validate and verify the Ideal BVM bioreactor system functionality and use. The Ideal BVM incorporated a user friendly design and assembly was simple and straightforward. The scaffold was easy to insert without the need for bending or twisting. The leak-proof chamber was easy to seal and permitted quick and sterile assembly in the hood. Scaffold length and diameter were adjustable for greater testing variability. The chamber was optically clear allowing for easy visibility of scaffold, media and stent placement and was sterilizable by ethylene oxide (EO) gas. The Ideal BVM chamber was easy to manufacture without the need for adhesive or special tooling and was constructed of materials and components that were readily available and easy to obtain resulting in a low cost design that was easy to scale up. Multiple chambers could be configured in the incubator and the use of biocompatible materials allowed cells to grow and function.

These findings support the hypothesis that design changes could be made and new configurations implemented to the original BVM system that would improve function and usability for preclinical device testing. Specifically, the work described in this chapter supports the hypothesis that a BVM chamber could be created which has the advantages of easy graft insertion and seal-ability, as well as the ability to accommodate

varying sizes of vessel scaffolds, all while maintaining the needs of a tissue engineering bioreactor system.

Several other bioreactor systems currently exist for development of tissue engineered blood vessels (TEBV). Most of these systems, similar to the Ideal BVM system, use a peristaltic pump to create flow within an optically clear chamber. However, chamber design, scaffold orientation, and general configuration vary considerably. For example, a custom designed vertical bioreactor chamber was built which housed a non-compliant silicone scaffold seeded with endothelial cells (EC) to examine the effects of shear stress and intravascular pressure (Punchard, et al. 2007). A second design utilized a large laboratory flask with seeding and cultivation of vascular smooth muscle cells (VSMC) on a PLCL [poly-(lactide-co-caprolactone)] scaffold oriented vertically within the chamber (Jeong, et al. 2005). These vertical bioreactor designs are shown below in Figure 3.36. Two other designs which examined the effects of pulsatile flow on smooth muscle and endothelial cell development utilized bioreactor systems that were very similar to the Ideal BVM system, with horizontal scaffold orientation (Hoerstrup, et al. 2001; Engbers-Buijtenhuijs, et al. 2006). These systems were assembled so that many scaffolds could be cultivated at once. Figure 3.37 shows the basic schematics of these two horizontal systems. In comparison with these bioreactor systems, the Ideal BVM differed in that it was designed for the purpose of preclinical testing. Therefore, separate individual flow paths were needed for each system and the ability to scale-up was a large focus. However, in general, each bioreactor system for the development of tissue engineered blood vessels must be designed so that sterility is maintained at all times and flow can be created through a scaffold lumen. Designs differ based on the available

resources, desired features, purpose of the study, and designer ingenuity. In the case of the Ideal BVM system specific criteria were set in advance which guided the overall design.

Figure 3.36- Vertical bioreactor chambers.

(Permission granted to reprint image from Punchard, et al. 2007 published in Journal of Biomechanics by Elsevier Limited.; Permission granted to reprint image from Jeong, et al. 2005 published in Biomaterials by Elsevier Limited.; see Appendix G)

Figure 3.37- Horizontal bioreactor systems.

(Permission granted to reprint image from Hoerstrup, et al. 2001 published in European Journal of Cardio-Thoracic Surgery by Elsevier Limited.; Permission granted to reprint image from Engbers-Buijtenhuijs, et al. 2006 published in Biomaterials by Elsevier Limited.; see Appendix G)

An additional advantage of the Ideal BVM system is that the basic design used to create the inlet and outlets of both the Ideal BVM chamber and two-port media reservoir can be easily applied to a wide range of container shapes and sizes. Depending on need and desired scaffold configuration holes can be placed in any location within the container. For example, a larger container could allow for longer scaffold lengths, or placement of inlet and outlet ports in varying configurations could accommodate more physiologic scaffold orientations, such as bends and bifurcations. Also, if additional ports are desired for injection of media into the space surrounding the scaffold, they can be easily added at any location desired. It is important to note that as container length and

size increase so does container volume. Volume of the BVM chamber is not a critical factor, but does affect the amount of media needed to fill the system and should be considered prior to implementing a different container size.

Given the possible variability of the general Ideal BVM chamber design, the following chapter will explore the potential to create alternate chambers for the evaluation of more complex vascular devices as well as the creation of chambers that accommodate more physiological vascular models. This idea of next generation BVM systems will be explored as potential future uses of the *in vitro* blood vessel mimic for preclinical device testing.

4. DESIGN OF NEXT GENERATION BVMS

Introduction

A BVM system can allow initial assessments to be performed on a large number of coating combinations and stent configurations in order to direct animal and clinical studies toward the most promising devices. The aim of the work in the previous chapters was to improve upon the original BVM system by developing a new, more ideal design and to describe in detail the finalized design, construction and setup of the Ideal BVM system and the testing performed to validate and verify its functionality and use.

The design of both the original BVM and Ideal BVM systems focused on straight, simple scaffold orientations representing simple lesions and single vessel disease (Fournier, et al. 2005). Coronary stent implantation, however, is being performed in an increasing number of patients with a wide spectrum of clinical and lesion characteristics. The anatomical characteristics of coronary arteries vary considerably, ranging from straight simple geometries to extremely angulated bends and curves as well as bifurcations and long lesions (Ellis, et al. 1990; Zhu, et al. 2008; Smith, et al. 2001). Depending on the individual, the anatomical structure of the coronary arteries can include all or some of the characteristics identified above. These lesion characteristics, including long lesions, angulated geometries, and bifurcations, will be described in detail below and will provide an introduction to the aims of this chapter.

The Ideal BVM system was designed to test stents ranging from 2.0-5.0 mm diameters and 8-38 mm lengths (Kandzari, Tchong and Zidar 2002; Kraitzer, Kloog and Zilberman 2008), in order to accommodate the variations of coronary arteries observed in

clinical practice and the stents currently available in the market. However, single coronary lesions are often longer than 38 mm, sometimes greater than 60 mm in length, and treatment requires multiple overlapping stents (Ruchin, et al. 2008). Using the BVM system for preclinical testing of overlapping stents could allow for better understanding of the cellular response at the overlapping junctions as well as the effect of the potentially double local drug dose when using drug-eluting stents (Kang, et al. 2007). In addition, new interlocking stent designs, such as the Custom NX(R) DES System manufactured by XTENT, Inc., are exploring the possibility of deploying stents up to 60 mm in total length (Stella, et al. 2008). Therefore, a BVM chamber for the preclinical testing of overlapping and longer total stent lengths would be beneficial.

According to the American College of Cardiology/American Heart Association (ACC/AHA) Guidelines for Percutaneous Coronary Intervention, non-angulated coronary artery geometries are defined as bends that are less than 45°, while moderately angulated geometries represents bends between 45° and 90°, and extremely angulated geometries are considered bends in excess of 90° (Smith, et al. 2001). A variety of angled geometries are illustrated below in Figure 4.1. Figure 4.2 is an X-ray angiogram image of the coronary arteries and shows the variation of curvatures observed in clinical practice.

Figure 4.1- Coronary artery geometries. Non-angulated bends are between 0° (a) and 45° (b), moderately angulated bends are between 45° and 90° (c), and extremely angulated geometries are greater than 90° . Examples of extremely angulated geometries are shown as 135° (d) and 180° (e).

Figure 4.2- X-ray angiogram image of the coronary arteries. Various angled bends and curvatures are present.

(Permission granted to reprint image from Xu, et al. 2007 published in Computer Methods and Programs in Biomedicine by Elsevier Limited.; see Appendix G)

As mentioned previously, the Ideal BVM system was designed for straight, simple scaffold orientations representing simple lesions and ideal anatomical geometry. In clinical practice, however, coronary arteries are not straight and uniform. They run along the surface of the heart and are curved, bent, and angulated (Smith, et al. 2001). In addition, it has been observed that plaque accumulation is related to vessel curvature, with plaque accumulating more rapidly in angulated vessels where wall shear stress is low (Wahle, et al. 2006; Zhu, et al. 2008). As a result, stents are most often placed in curved positions within the coronary arteries and remain in a bent position throughout their lifetime (Fournier, et al. 2005; Smith, et al. 2001). Many coronary stents, such as the MULTI-LINK manufactured by Abbott Vascular, CYPHER[®] manufactured by Cordis,

and TAXUS[®] manufactured by Boston Scientific, also claim superior flexibility and conformity which allow the stent to mold to the natural contours of the vessel wall (Serruys and Gershlick 2005; Rieu, et al. 2003). Therefore, a BVM chamber for the preclinical testing of devices in curved and angulated vessels would be beneficial and would represent a more physiological blood vessel mimic model.

As described by the ACC/AHA a coronary bifurcation occurs when one main vessel branches out into two smaller vessels, one being the continuation of the main vessel, and the other referred to as the side branch (Smith, et al. 2001). A coronary bifurcation lesion is defined as a narrowing involving both the main vessel and a significant side branch (Louvard, et al. 2008). Illustrations of two bifurcated geometries, Y-shaped and T-shaped, are shown below in Figure 4.3. Figure 4.4 is an X-ray angiogram image of Y-shaped and T-shaped bifurcations observed in clinical practice.

Figure 4.3- Bifurcated coronary artery geometries. Y-shaped (a) and T-shaped (b).

Figure 4.4- X-ray angiogram image of bifurcated coronary arteries. Y-shaped (a) and T-shaped (b).

(Permission granted to reprint image from Ikeno, Buchbinder and Yeung 2007 published in Cardiovascular Revascularization Medicine by Elsevier Limited.; see Appendix G)

Bifurcation lesions account for 15-18% of all coronary lesions and represent one of the most complex coronary lesions to treat since the lumen of both the main vessel and side branch are affected and require treatment (Sukhija, Mehta and Sachdeva 2008). Recent advances have allowed physicians to treat bifurcation lesions, where both the main vessel and side branch need to be restored. Many techniques, with the use of one or multiple stents, have been developed to treat bifurcation lesions (Sukhija, Mehta and Sachdeva 2008; Iakovou, Ge and Colombo 2005). The simplest approach is to stent the main vessel and dilate the side branch through the stent struts. A second approach is to use multiple stents and can be accomplished through a number of different techniques which were discussed in Chapter 1: V-stenting, simultaneous kissing, crush, T-stenting, modified T-stenting, Y-stenting, and skirt. Most recently, novel stents and delivery

systems such as AXXESS™ manufactured by Devax, Inc., PETAL™ manufactured by Boston Scientific, and MULTI-LINK Frontier manufactured by Abbott Vascular have been developed specifically to treat bifurcation lesions (Grube, et al. 2007; Ikeno, Buchbinder and Yeung 2007; Miyazawa, et al. 2007; Lefèvre, et al. 2005). Therefore, a BVM chamber for the preclinical testing of devices in bifurcation shaped vessels would be beneficial and would support advancing technology.

In summary, the variation and complexity of coronary artery disease and the advanced capabilities to detect and determine the extent and severity of coronary lesions has led to an increased interest in intravascular device innovation (Kini 2006). Stents are most often placed in vessels with angulated geometries and remain in bent positions throughout their lifetime (Fournier, et al. 2005; Smith, et al. 2001). Long lesions can require two overlapping stents (Ruchin, et al. 2008) and bifurcation lesions often require two stents at varying angles or the use of a novel stent specifically designed for a bifurcation (Sukhija, Mehta and Sachdeva 2008; Ikeno, Buchbinder and Yeung 2007). Thus, the aim of the work in this chapter was to expand the BVM concept to meet the need of future technologies that currently have not been adequately addressed in preclinical models, such as long, angulated and bifurcation lesions. The hypothesis was that the need to test stents specifically for long, angulated and bifurcation lesions can be potentially addressed by using modified BVM systems. Although full implementation and testing of these models is beyond the scope of this thesis, the current work will explore the development of new and innovative models for the evaluation of more complex lesions as well as the creation of chambers that accommodate more physiological vascular models and scaffold orientations. Therefore, this chapter will

explore the creation of alternate chambers for the evaluation of more complex vascular devices as potential future uses of the *in vitro* blood vessel mimic for preclinical device testing.

Next Generation BVM System Designs

BVM System for Long Vessels

A BVM system for long vessels can be created by using a longer chamber, such as the 800 ml, 8" x 5" x 2", semitransparent, polypropylene rectangular snap and seal leak-proof food container (Lock & Lock P/N HPL816) and implementing the same design and construction described in the previous chapter and detailed in Appendix C for the assembly of the luminal inlet and luminal outlets. Locations of the luminal inlet and luminal outlets are identical to the Ideal BVM system. A chamber of this size can accommodate scaffold lengths up to 170 mm which would allow long or multiple stents to be placed and overlapped within the vessel lumen. It is important to note that the volume of this chamber is greater than that of the Ideal BVM system. Volume of the BVM chamber is not a critical factor, but does affect the amount of media needed to initially fill the system and should be considered prior to implementation. The BVM system for long vessels is modeled below in Figures 4.5 and 4.6.

Figure 4.5- BVM system for long vessels.

Figure 4.6- Scaffold setup for long vessels. Longer scaffold lengths can be accommodated in a modified BVM system by using a longer chamber.

Scaffold setup and insertion for the BVM system for long vessels will remain unchanged from the assembly process used for the Ideal BVM system described in the previous chapter, except for the length of ePTFE scaffold used. As in the previous design, male luer integral lock ring to female luer thread style couplers can be used to vary the overall scaffold length. A prototype of this model would be needed to establish the corresponding scaffold length versus number of couplers used.

The design of the BVM system for long vessels is almost identical to the Ideal BVM system and implements a chamber that is identical except for length and volume. As a result the BVM chamber for long vessels meets all of the design criteria established in the previous chapters.

BVM System for Angulated Vessels

A BVM system for angulated and curved vessels can be created by varying the placement of the luminal inlet and luminal outlet holes on the Ideal BVM chamber. Construction of the luminal inlet and luminal outlet holes can be accomplished using the same materials and methods described in the previous chapter and detailed in Appendix C. Prototypes must be built in order to determine the exact dimensional location and placement of the luminal inlet and luminal outlet holes. However, the illustrations below approximate their general locations. A large variation of curves and angles can be created ranging from slight bends to 180° turns, representing non-angulated, moderately angulated and severely angulated geometries. Construction of several modified BVM chambers for angulated vessels are described below. All designs utilize the same chamber as the Ideal BVM system, a 350 ml, 4" x 5" x 2", semitransparent, polypropylene rectangular snap and seal leak-proof food container (Lock & Lock P/N HPL806).

The simplest bend that can be created requires only one change to the tubing configuration of the Ideal BVM system. Locations of the luminal inlet and luminal outlet holes are identical to the Ideal BVM system. By reversing the placement of the luminal outlet and extra luminal outlet and connecting the scaffold as described in the previous chapter, a slightly bent scaffold can be accommodated. This setup represents a non-angulated geometry and can accommodate scaffold orientations with bends of 45° or less. The BVM system for slightly bent vessels is modeled below in Figures 4.7 and 4.8.

Figure 4.7- BVM system for slightly bent vessels.

Figure 4.8- Scaffold setup for non-angulated vessels. A slightly bent scaffold can be accommodated in a modified BVM chamber by reversing the luminal outlet and extra luminal outlet positions.

The second bend that can be created requires only a slight modification to the configuration of the Ideal BVM system. By moving the luminal outlet and extra luminal outlet to the long side of the chamber and connecting the scaffold as described in the

previous chapter, a moderately angled scaffold can be accommodated. This setup represents moderately angled geometry and can accommodate a scaffold orientation with a bend of 90° . The BVM system for moderately angled vessels is modeled below in Figures 4.9 and 4.10.

Figure 4.9- BVM system for moderately angled vessels.

Figure 4.10- Scaffold setup for moderately angled vessels. A moderately angled scaffold of 90° can be accommodated in a modified BVM chamber by moving the luminal outlet and extra luminal outlet to the long side of the chamber.

The third bend that can be created requires a minor modification to the configuration of the Ideal BVM system. By moving the luminal inlet, luminal outlet and extra luminal outlet to the long side of the chamber and connecting the scaffold as described in the previous chapter, a severely angled scaffold can be accommodated. This setup represents severely angled geometry and can accommodate a scaffold orientation with a bend of 180° . The BVM system for severely angled vessels is modeled below in Figures 4.11 and 4.12.

Figure 4.11- BVM system for severely angled vessels.

Figure 4.12- Scaffold setup for severely angulated vessels. A severely angled scaffold of 180° can be accommodated in a modified BVM chamber by moving the luminal inlet, luminal outlet and extra luminal outlet to the long side of the chamber.

Scaffold setup and insertion for the BVM systems for angulated vessels will remain unchanged from the assembly process used for the Ideal BVM system described in the previous chapter, except for the lengths of ePTFE scaffolds used. As in the previous design, male luer integral lock ring to female luer thread style couplers can be used to vary the overall scaffold lengths in each design. Prototypes of each model are needed to establish the corresponding scaffold lengths versus number of couplers.

One challenge that may be encountered in these configurations is kinking and occlusion of the scaffolds due to their bent orientations. However, ePTFE material is flexible and compliant and once flow is created via a peristaltic pump, the force on the scaffold walls created by the pressure of the media may be strong enough to overcome any kinks or occluded bends that occur. This hypothesis will need to be verified and tested with full-scale prototypes. If kinking or occlusion proves to be a challenge a

change in the scaffold material or fabrication method may need to be addressed. Pre-forming the scaffold in a bent orientation by using a mold or customized model could provide a solution. A thin wire coiled around the outside of the ePTFE could also be used to control the curvature and orientation without disrupting the inner lumen.

The designs of the BVM systems for angulated vessels are almost identical to the Ideal BVM system and use the exact same chamber. As a result the BVM chambers for angulated vessels meet all of the design criteria established in the previous chapters.

BVM System for Bifurcation Vessels

A BVM system for bifurcation vessels can be created by varying the placement of the luminal inlet, luminal outlet, and extra luminal outlet holes and adding a second luminal outlet to accommodate the side branch lumen. Construction of the luminal inlet and luminal outlet holes can be accomplished using the same materials and methods described in the previous chapter and detailed in Appendix C. Prototypes must be built in order to determine the exact dimensional location and placement of the luminal inlet and luminal outlet holes. However, the illustrations below approximate their general locations. The most notable change is the modified tubing configuration which is necessary to accommodate flow through the second luminal outlet. Construction of two modified BVM chambers for Y-shaped and T-shaped bifurcation vessels are described below. Each design utilizes the same chamber as the Ideal BVM system, a 350 ml, 4" x 5" x 2", semitransparent, polypropylene rectangular snap and seal leak-proof food container (Lock & Lock P/N HPL806).

The first bifurcation geometry that can be created requires some modifications to the configuration of the Ideal BVM system. By moving the extra luminal outlet to the long side of the chamber and adding a second luminal outlet in its place a Y-shaped bifurcation scaffold can be accommodated. A Y-shaped bifurcation scaffold can be setup and connected as described in the previous chapter with the addition of one female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1). One male-to-male luer lock connector, with one rotating collar (Qosina P/N 17644) also needs to be attached to the second outlet to hold the scaffold. The easiest way to configure the tubing is to simply add a second Y-connector with 200 series barbs, 1/8" ID tubing (Value Plastics P/N Y230-1) prior to the attachment of the one-way stopcock. In addition, a second one-way stopcock (Value Plastics P/N VP455980) must be added to the second luminal outlet to control flow. This modified orientation represents Y-shaped bifurcation geometry and can accommodate a Y-shaped scaffold. The BVM system for Y-shaped bifurcation vessels is modeled below in Figures 4.13 and 4.14.

Figure 4.13- BVM system for Y-shaped bifurcation vessels.

Figure 4.14- Scaffold setup for Y-shaped bifurcation vessels. A Y-shaped bifurcation scaffold can be accommodated in a modified BVM chamber by moving the extra luminal outlet to the long side of the chamber and adding a second luminal outlet in its place.

The second bifurcation geometry that can be created requires one simple modification to the configuration of the Ideal BVM system. By adding a second luminal outlet to the long side of the chamber a T-shaped bifurcation scaffold can be accommodated. A T-shaped bifurcation scaffold can be setup and connected as described in the previous chapter with the addition of one female luer thread style to 200 series barb, 1/8" ID tubing (Value Plastics P/N FTLL230-1). One male-to-male luer lock connector, with one rotating collar (Qosina P/N 17644) also needs to be attached to the second outlet to hold the scaffold. The easiest way to configure the tubing is to simply add a second Y-connector with 200 series barbs, 1/8" ID tubing (Value Plastics P/N Y230-1) prior to the attachment of the one-way stopcock. In addition, a second one-way stopcock (Value Plastics P/N VP455980) must be added to the second luminal outlet to

control flow. This modified orientation represents T-shaped bifurcation geometry and can accommodate a T-shaped scaffold. The BVM system for T-shaped bifurcation vessels is modeled below in Figures 4.15 and 4.16.

Figure 4.15- BVM system for T-shaped bifurcation vessels.

Figure 4.16- Scaffold setup for T-shaped bifurcation vessels. A T-shaped bifurcation scaffold can be accommodated in a modified BVM chamber by adding a second luminal outlet to the long side of the chamber.

Scaffold setup and insertion for the BVM systems for bifurcation vessels will remain unchanged from the assembly process used for the Ideal BVM system described in the previous chapter. However, Y-shaped and T-shaped bifurcation ePTFE scaffolds will need to be constructed. Sewing standard ePTFE material together in the desired shape could provide a feasible method and is illustrated in Figures 4.17 and 4.18 below.

Figure 4.17- Y-shaped bifurcation scaffold. Constructed by sewing three short pieces of ePTFE material together.

Figure 4.18- T-shaped bifurcation scaffold. Constructed by creating a hole in the center of a long piece of ePTFE material and sewing a short piece of ePTFE material to the hole.

One challenge that may be encountered with this assembly process is leakage at the suture sites. Prototype scaffolds will need to be constructed and inserted into the BVM chambers and flow via a peristaltic pump will need to be initiated to evaluate the potential leakage. Using a mold or customized model to form the entire scaffold in a bifurcation shape could also provide a solution and would eliminate the possibility of leakage, although a material other than ePTFE would need to be chosen and evaluated. As in the previous design, male luer integral lock ring to female luer thread style couplers can be used to vary the overall scaffold lengths in each design. Prototypes of each model are needed to establish the corresponding scaffold lengths versus number of couplers.

The designs of the BVM systems for bifurcation vessels are very similar to the Ideal BVM system and use the exact same chamber. As a result the BVM chambers for bifurcation vessels meet all of the design criteria established in the previous chapters.

Next Generation BVM System Prototypes

Six modified BVM systems representing complex coronary vessel geometries were modeled above: long, slightly bent, moderately angulated, severely angulated, Y-shaped bifurcation and T-shaped bifurcation. Although full implementation and testing of these models is beyond the scope of this thesis, a multipurpose and Y-shaped bifurcation BVM system prototype were built with the intention of verifying the feasibility of these designs. The only configuration that was not prototyped was the BVM system for long vessels, since the configuration is identical to the Ideal BVM system with the exception of a longer chamber.

Multipurpose BVM System

Instead of building each individual BVM system prototype, a multipurpose BVM system was built with appropriate luminal inlets and luminal outlets to accommodate complex vessel configurations. This modified BVM chamber accommodated slightly bent, moderately angulated, severely angulated, and T-shaped bifurcation configurations. The luminal inlets and luminal outlets that were not required for a specific configuration were simply occluded using a male luer integral lock ring plug (Value Plastics P/N LP4-1). As proposed, two pieces of ePTFE material were sutured together to make the T-shaped bifurcation scaffold. The multipurpose BVM system prototype, with a slightly bent scaffold, a moderately angulated 90° scaffold, a severely angulated 180° scaffold, and a T-shaped bifurcation scaffold are shown below in Figures 4.19 through 4.22.

Figure 4.19- Multipurpose BVM System with a slightly bent scaffold.

Figure 4.20- Multipurpose BVM System with a moderately angulated 90° scaffold.

Figure 4.21- Multipurpose BVM System with a severely angulated 180° scaffold.

Figure 4.22- Multipurpose BVM System with a T-shaped bifurcation scaffold.

Y-Shaped Bifurcation BVM System

A Y-shaped bifurcation BVM system was built with appropriate luminal inlets and luminal outlets to accommodate a Y-shaped bifurcation vessel configuration. However, instead of suturing three separate piece of ePTFE together, as purposed, a simpler method was used. Due to the flexibility of ePTFE material and the desire to use as few sutures as possible, the arms of the T-shaped scaffold were bent upwards to create a Y-shaped scaffold. This method eliminated the need for three separate ePTFE pieces to be sutured together, reducing the chance of leakage at the suture points. The Y-Shaped Bifurcation BVM system prototype is shown below in Figure 4.23.

Figure 4.23- Y-Shaped Bifurcation BVM System with a Y-shaped bifurcation scaffold.

Discussion and Conclusions

The next generation BVM systems, presented through computer-generated models and initial prototypes, represent the development of new and innovative designs for the evaluation of more complex lesions that currently have not been adequately addressed in preclinical models and support the hypothesis that the Ideal BVM system can be modified to accommodate advancing technology. The work in this chapter expanded the BVM concept to meet the needs of long, angulated and bifurcation lesions. Alternate chambers for the evaluation of more complex vascular devices, as potential future uses of the *in vitro* blood vessel mimic for preclinical device testing, were illustrated and prototyped.

Full implementation and testing of the modified BVM systems was beyond the scope of this thesis. Additional prototypes and verification testing is needed to demonstrate that the modified BVM systems developed in this chapter meet the testing needs for long, angulated and bifurcation lesions. The prototypes of each modified BVM chamber need to be finalized and the entire bioreactor system needs to be assembled and connected to a peristaltic pump. Scaffolds for each system also need to be assembled, measured and connected to the modified BVM systems to establish lengths, and evaluate the possibility for kinking or occlusion due to bent orientations. Bifurcation scaffolds will need to be constructed and evaluated for their ability to function under pressure and flow conditions.

The first step in the evaluation of each model requires full construction of the modified BVM systems. Construction of the modified BVM chambers utilize the same

materials and methods described in the previous chapter and detailed in Appendix C. Location of the luminal inlets and outlets vary between models, but the assembly process remains unchanged. The construction of a multipurpose and Y-shaped bifurcation BVM chamber prototype and the assembly of each complex vessel scaffold configuration within these chambers were shown, verifying the feasibility of these designs.

The complete bioreactor configurations, although not illustrated in this chapter, consist of the modified BVM systems and two-port media reservoirs, described in the previous chapter, connected in series. Setup is identical to the Ideal BVM bioreactor configuration and only two simple connections need to be made. Specific placement of male luer lock and female luer thread fittings ensure proper setup and orientation every time. Only one configuration exists for setting up the modified BVM bioreactor systems, since the fittings of all modified BVM chambers and two-port media reservoirs were designed as male-to-female connections.

The luminal inlet, luminal outlets and extra luminal outlet tubing configurations are identical to the Ideal BVM system and permit variation in flow path. Transluminal and transmural flow are easily controlled via the one-way stopcocks attached to the luminal outlets. For the bifurcation models, one-way stopcocks are attached to both the main vessel luminal outlet and the side branch luminal outlet, allowing control of flow through each of the bifurcation paths. By closing off the luminal outlet paths, through the use of the one-way stopcocks, transmural flow through the porous ePTFE scaffold walls is created, with flow exiting the extra luminal outlets. Transmural flow is used for conditioning and cell seeding of the scaffold material. By opening the one-way

stopcocks, transluminal flow is restored through the luminal outlets. The one-way stopcocks are easy to use and provide a quick and simple method for controlling the flow paths. The levers are easy to turn and once in position flow is immediately and firmly blocked. The merging of the luminal outlets and extra luminal outlets into one main outlet via Y-connectors make attachment to the two-port media reservoir simple with the need for only one connection. The three-way stopcocks attached to the luminal inlets easily function as both flow paths and cell sodding ports. With two female luer thread fittings, the three-way stopcocks allow the modified BVM systems to be connected to the two-port media reservoirs as well as maintain an extra port for cell sodding and media injection.

Few models, other than animal and clinical studies, exist for preclinical testing of complex intravascular devices designed specifically for placement in complex coronary lesion geometries. Currently most preclinical biocompatibility and tissue response testing is done on animals in outside laboratories which is both time consuming and expensive, especially when testing involves device placement and orientation in long, angulated and bifurcation vessels. Although preclinical animal studies are accepted as a means to predict the probability of device safety and effectiveness in humans, the need exists for preclinical testing using live human tissue. Preclinical testing using human cells would greatly increase the medical community's understanding of tissue response to complex intravascular devices prior to animal and patient testing and prior to device release into the market. Presently, it is difficult to simulate device and tissue interaction and research still needs to be done to fully understand tissue behavior and the *in vivo* interaction that occurs between device and tissue in complex coronary lesion geometries.

In order to address some of these limitations, there has been research on the use of alternate or *in vitro* models for this type of complex device testing. One research group that wanted to evaluate the use of coronary stents in the side branch of a bifurcation lesion constructed a testing model made of Plexiglas with a 4.5 mm bifurcation shaped trough bored out of the center in order to simulate a bifurcation vessel (Ormiston, et al. 1999). This type of synthetic model approach is not uncommon, and the model does allow stents to be deployed and evaluated in a bifurcated geometry, however no cellular lining exists to provide physiological response evaluations. A second research group that wanted to evaluate the response of endothelial cells to flow hemodynamics at an arterial bifurcation apex constructed a T-shaped bifurcation *in vitro* flow chamber (Szymanski, et al. 2008). This chamber was custom designed and constructed of polycarbonate, stainless steel, and nylon, which are all autoclavable materials. This model, unlike the previous model, was constructed for the purpose of establishing a cellular lining at the bifurcation apex. However, intravascular devices were not deployed in this model and it is not clear if the design allows for this type of evaluation.

With increasing complexity of coated stents and devices that interface with cells and tissue (Waksman 2007; Waksman 2002; Sharkawi, et al. 2007) the ability to evaluate in a living environment is crucial. The modified BVM systems developed in this chapter were designed to support the growth of 3-dimensional *in vitro* tissue engineered blood vessel mimics in order to provide physiological models of complex coronary geometries. With advancing technology these modified BVM systems may one day provide an ideal environment for evaluation of complex vascular devices and direct animal and clinical studies toward the most promising advances.

5. DISCUSSION AND CONCLUSIONS

To summarize, the work in this thesis describes the design and optimization of an *in vitro* blood vessel mimic (BVM) bioreactor system for the evaluation of intravascular devices, specifically coronary stents, in simple and complex vessel geometries. The general hypothesis driving this thesis project was that design changes could be made and new configurations implemented to the existing blood vessel mimic system (Cardinal, et al. 2006) that would improve function and usability for preclinical device testing. In order to test this hypothesis, research was conducted based on two specific aims. The first aim was to develop a more ideal BVM system. The specific hypothesis in this case was that a BVM chamber could be created to accommodate a wider range of stent lengths and diameters, while allowing for easy graft insertion and seal-ability. The second aim was to develop next generation BVM systems, focused on future needs and technology. The hypothesis in this case was that with emerging technology the ability to test stents specifically for bifurcation lesions, as well as in long and angulated vessels, will be needed and is possible within the BVM system. The prototypes developed and the findings from constructed models were presented and discussed in the previous chapters and support the hypotheses stated above.

The more ideal BVM bioreactor system designed and developed in this thesis was successfully built and implemented. A more user friendly design was one of the primary needs met by the Ideal BVM system. Easy scaffold insertion, without the need for bending or twisting, along with a method to quickly and simply seal the vessel chamber without the need for tools, greatly improved the usability of the BVM system. As

discussed in detail in the introduction to this thesis, stents range in shape and size, and the Ideal BVM system was designed to accommodate stent sizes ranging from 2.0-5.0 mm diameters and 8-38 mm lengths in order to fit the variations of coronary arteries observed in clinical practice and the stents currently available in the market. In addition, the Ideal BVM chamber was easy to manufacture without the need for adhesive or special tooling and was constructed of materials and components that were readily available and easy to obtain resulting in a low cost design that was easy to scale up. Multiple chambers could be configured in the incubator and the use of biocompatible materials allowed cells to grow and function. Therefore, the work described in this thesis supports the use of the Ideal BVM system for cultivation of *in vitro* blood vessel mimics.

The next generation BVM systems, presented in this thesis through computer-generated models and initial prototypes, represent the development of new and innovative designs for the evaluation of more complex lesions that currently have not been adequately addressed in preclinical models. The work presented supports the hypothesis that the Ideal BVM system can be modified to accommodate advancing technology. Alternate chambers for the evaluation of more complex vascular devices, as potential future uses of the blood vessel mimic system for preclinical device testing, were illustrated and prototyped. Full implementation and testing of the modified BVM systems was beyond the scope of this thesis. However, the construction of a multipurpose and Y-shaped bifurcation BVM chamber prototype and the assembly of each complex vessel scaffold configuration within these chambers were shown, verifying the feasibility of these designs. With advancing technology, these modified BVM systems may one day

provide an ideal environment for evaluation of complex vascular devices in order to direct animal and clinical studies toward the most promising technologies.

The goal of this thesis was to not only expand upon the original BVM model, but to develop new and innovative models for the evaluation of devices in more complex geometries such as bifurcations, long and angulated vessels. The BVM systems developed in this thesis are model systems which were developed for the initial testing of newly emerging intravascular technology, and represent the next step in preclinical device testing. In the following sections, key aspects of this field and relevant areas of research will be discussed with regard to the present thesis work.

Preclinical Intravascular Device Testing

Preclinical device testing is a crucial step in the process of developing and releasing new medical devices to the market. The objective of preclinical testing is to provide preliminary evidence of product safety and efficacy in order to support the initiation of human clinical trials. Preclinical testing typically begins using models and methods that are simple and cost-effective in order to identify potential failures as early in the development process as possible, and then progresses to models with increasing complexity. As discussed in the introduction, preclinical intravascular device testing is currently performed using both *in vitro* laboratory models and *in vivo* animal models and provides critical information about cellular and tissue responses as well as device success.

Although preclinical animal studies are accepted as a means to predict the probability of device safety and effectiveness in humans, no animal model can provide complete insights into the human healing response following stent implantation. Preclinical testing using human cells would greatly increase the medical community's understanding of tissue response to a specific device prior to animal and patient testing and prior to device release. The Ideal BVM system developed in this thesis represents an intermediate testing environment for assessing human cellular responses to intravascular stents in a manner that combines the advantages of both *in vitro* and *in vivo* approaches. The Ideal BVM system mimics the geometries of the coronary arteries and utilizes human cells, which provide the potential for more predictive preclinical results than animal models alone can provide. The Ideal BVM system developed in this thesis

provides a feasible method to rapidly evaluate human tissue response to varying stent configurations, materials, drugs and coatings as they become available. Although the Ideal BVM system is not a substitute or replacement for animal models, it has the potential to provide researchers with a more relevant *in vitro* tissue model that can be used for the preclinical evaluation of new intravascular devices. The major advantage of the *in vitro* blood vessel mimic system developed in this thesis is its potential for consistent high-throughput evaluation of cellular response to advancing intravascular technologies (Cheng, Lai and Kisaalita 2008; Yliperttula, et al. 2008).

Laminar Flow and Wall Shear Stress

One area that has not yet been addressed as a potential use for the blood vessel mimic (BVM) bioreactor systems developed in this thesis is the study of laminar flow and wall shear stress in stented vessels. Implanted stents can alter coronary flow and induce significant changes in flow hemodynamics, especially in bifurcated and angulated geometries where flow is already disturbed and shear stress is low (Zhu, et al. 2008; Wahle, et al. 2006; Benard, et al. 2003). These changes result in local modification of the mechanical stress exerted on the endothelial cells lining the vessel wall and several studies have demonstrated that endothelial cell proliferation increases significantly when subjected to weak shear stress and non-laminar flow (Ridger, et al. 2008; Scott 2006).

A number of experiments have been performed to study the influence of an implanted stent on flow hemodynamics through a stented vessel (Peacock, et al. 1995; Benard, et al. 2003). However, most of these studies used computer generated

computational techniques rather than animal or *in vitro* physical models (Balossino, et al. 2008; Dehlaghi, Shadpoor and Najarian 2008). As a result, the influence of flow disturbances on the healing response to an implanted stent is poorly understood and the need exists for anatomically realistic testing models with cellular linings to obtain more predictive preclinical results.

The use of the BVM systems developed in this thesis to study flow hemodynamics in stented vessels could provide important information about the influence of flow and shear stress on vascular healing. The BVM system could be used to not only study the cellular response to laminar flow and shear stress changes due to the presence of a stent, but could provide a model that would simultaneously allow for direct flow and velocity measurements. The BVM bioreactor system created in this thesis is a closed system in which all physical parameters, including flow, can be tightly controlled and monitored while allowing visualization and access to the inner lumen of the stented vessel. These advantages make the *in vitro* BVM system an ideal model for the study of flow hemodynamics.

Drug Elution Kinetics

Blood flow through a stented vessel not only affects laminar flow and wall shear stress, but plays a direct role on drug elution kinetics, or drug release, from drug eluting stents (Umashankar, Hari and Sreenivasan 2007). As discussed in the introduction to this thesis, a drug eluting stent is a device that releases a single or multiple bioactive agents into the bloodstream that affect the tissue adjacent to the stent. Currently there are three

different approaches to binding drugs to coronary stents: drug binding by means of a polymer on the stent surface, drug binding aided by an inorganic stent coating, or direct drug binding to the stent surface without coating (Acharya and Park 2006). Most often drugs are either chemically bonded to the surface of the stent or trapped within a polymer film or porous ceramic that allows controlled drug release (Kandzari, Tcheng and Zidar 2002). Drug release in biodegradable coatings is achieved through disintegration of the biodegradable polymer of the stent, while non-biodegradable coatings release drugs by allowing the drugs to diffuse through a porous matrix. Whatever mechanism of drug binding used flow characteristics can have a significant influence on drug release and distribution within the tissue of the stented vessel (Neubert, et al. 2008).

Currently, most preclinical drug elution studies consist of immersion of the drug eluting stent in a small volume of liquid, with drug concentration measurements taken over time or the use of computer generated computational models (Neubert, et al. 2008; Balakrishnan, et al. 2007; Alexis, et al. 2004). These methods allow for the examination of drug release but lack simulation of the vessel wall and cellular response and their possible influence on drug release and distribution. The examination of *in vivo* release and distribution is also limited since the drug concentrations in animals and humans cannot reliably be estimated due to low systemic blood concentrations and the fact that blood levels do not necessarily correspond to drug concentration at the stented lesion (Neubert, et al. 2008). Due to these limitations an *in vitro* model would be extremely valuable for the preclinical evaluation of drug elution kinetics.

The use of the *in vitro* BVM system developed in this thesis could provide a beneficial model for the study of drug elution kinetics. The BVM system provides an environment that could allow for observation and measurement of drug release and distribution within the vessel. With the addition of a cellular layer, the cellular response to drug concentrations in the blood and tissue could be better understood and a closed bioreactor system would permit precise drug concentration measurements at specific points in time. Drug concentrations in the BVM system could be easily measured in the circulating media, as well as in the media surrounding the scaffold, and in the scaffold and cellular layer itself. These types of measurements could provide important information on the influence of living tissue to drug release, distribution and local tissue affects which in turn could provide insight into the role of drug eluting stents on the cellular healing response and restenosis.

Delivery Systems

Much of this thesis has focused on the use of the BVM system to evaluate tissue response to implanted stents. However, as discussed in the introduction, in addition to the advancements in coronary stent design, the method of delivering these therapies has also improved. These improvements have made percutaneous coronary intervention procedures easier to perform and have reduced the amount of time for a single procedure.

Percutaneous coronary intervention in complex coronary geometries is difficult and the increased complexity results in reduced success rates (Saeed, Banerjee and Brilakis 2008). Therefore, novel delivery systems, smaller catheters, lower profile

balloons and other devices have been created to overcome the challenges associated with tortuous anatomies. Stent delivery is an area of active research and a living model system for testing and evaluating these new delivery devices would be ideal for development and progression of the field.

The future of the BVM system developed in this thesis has the potential to be used not only to evaluate tissue response to stent implantation but to the stent delivery processes and systems themselves. The BVM system could allow researchers to quickly evaluate the ability of the delivery system to safely and effectively implant a stent into a coronary vessel, with the advantage of observing tissue response to the delivery device, as well as the implanted stent.

The BVM Concept for Other Tissues

Given the possible variability of the general BVM system concept, the following section will explore the potential to create chambers for the evaluation of medical devices in varying physiological tissue models. The BVM system developed in this thesis was designed to mimic coronary blood vessels and vascular tissue. However, the general concept can be used to model any tube like structure in which flow is present and has the potential to provide *in vitro* models of a wide variety of human tissues.

Urology

The ureter is a narrow muscular tube in which urine flows from the kidney to the bladder. A ureter may become obstructed as a result of a number of conditions including strictures, inflammation, cancer and kidney stones. When the ureter is obstructed, urine is unable to drain from the kidney and leads to pain, infection and if left untreated, kidney failure. Therefore, action must be taken to restore the flow of urine to the bladder.

Ureteral stenting represents a minimally invasive procedure in which a ureteral stent, a small diameter plastic tube with pigtail shaped ends, is temporarily placed between the kidney and bladder to restore the flow of urine and provide a physical scaffold for the healing ureter. Ureteral stents have widespread use in current urological practice and are used for a wide variety of indications, the most common being the relief of urinary stone disease. Ureteral stents are used to maintain ureteral patency after endourological procedures and relieve ureteral obstruction.

One significant complication associated with ureteral stent placement is encrustation within the stent lumen, blocking the flow of urine (Laube, et al. 2008; Tunney, Keane and Gorman 1997). Infection and pain are also common complications. As a result a number of new ureteral stent designs, materials, and coatings have been introduced to the market with the goal of preventing bacterial buildup and encrustation while increasing patient comfort (Kleinen, Böde and Laube 2008; Wignall, et al. 2008; Cadieux, et al. 2006; Hafron, et al. 2006).

Several *in vitro* models have been developed for the preclinical testing of ureteral stents (Gorman, et al. 2003; Choong, Wood and Whitfield 2000; Stoller, et al. 2000). These models allow ureteral stents to be deployed and evaluated in anatomical geometries; however, no cellular lining exists to provide physiological response evaluations. Through the creation of an *in vitro* tissue engineered ureter construct a model system could be established for the initial testing of newly emerging urological technology. The need exists for new and advanced preclinical urological testing models that provide medical device manufacturers and researchers with the ability to quickly evaluate tissue response to new ureteral stent designs, materials and coatings.

Abdominal Aortic Aneurysm

Abdominal aortic aneurysm (AAA) is a common vascular disorder affecting approximately 5% of the male population over 55 years of age (Chong and How 2004). An abdominal aortic aneurysm occurs when a weak area of the abdominal aorta expands or bulges due to the high pressure of blood flowing through it. AAA is a significant

health risk since the aneurysm can burst or rupture at any time leading to severe bleeding, shock and death (Iribarren, et al. 2007). Therefore, action must be taken to ensure that if rupture or leakage occurs, blood flow through the abdominal aorta is not disrupted.

Until recently, open surgical replacement of the diseased aortic segment with a prosthetic graft was the standard treatment for AAA (Steingruber, et al. 2006; Chong and How 2004). Endovascular aortic aneurysm repair (EVAR), however, has become an increasingly successful minimally invasive procedure for treating AAA. During EVAR an endovascular stent-graft is permanently placed within the abdominal aorta at the site of the aneurysm. The stent-graft provides a path for blood to flow through the aneurysm, reducing the pressure exerted on the weakened walls.

There are significant complications associated with EVAR due to the high velocity flow and pressures present in the abdominal aorta. Complications include downstream migration of the stent-graft and leakage at the attachment site (Steingruber, et al. 2006). As a result development of the ideal endovascular stent-graft is ongoing and the need exists for an *in vitro* AAA flow model for evaluating endovascular fixation techniques, new stent-graft designs and materials, long term durability and the cellular response to these new technologies (Sulaiman, et al. 2008). Through the creation of an *in vitro* tissue engineered large diameter vessel construct a model system could be established for the initial testing of newly emerging AAA technology.

Potential as a Commercial Device

The BVM systems developed in this thesis have the potential to be created on a large scale and sold to the biomedical industry as commercial preclinical testing devices. The BVM system could be marketed as an *in vitro* bench-top testing apparatus that mimics the human coronary artery environment. The BVM system could allow medical device manufacturers and researchers to conduct their own individual preclinical biocompatibility testing on a number of designs using a predictive model which could reduce the number of animal studies required. The Multipurpose BVM, especially, has the potential to be an ideal preclinical model since simple and complex vessel scaffolds can be accommodated allowing researchers to test devices in a number of vessel geometries. This type of preclinical testing may lead to better understanding of device and tissue interaction much earlier in the development process and give researchers the chance to make design improvements prior to animal and clinical studies.

In order to market the BVM system as a commercial device, additional work would need to be completed. Extensive studies and comparisons would need to be made between the results obtained from the BVM system and animal models. Research would be needed to demonstrate that the BVM system provides predictive preclinical results that are comparable to those obtained from animal and clinical studies. Besides additional laboratory testing, ship testing would need to be performed to ensure that the BVM system can be transported without sustaining physical damage. In addition, testing would need to be conducted to ensure that the cells provided with the system are stored and packaged properly so that they survive shipment. Other considerations that would need to

be addressed include determining the number of times the system can be sterilized and reused prior to failure, the creation of detailed instructions for use, and large scale manufacturing ability.

Limitations and Future Work

The *in vitro* tissue engineered blood vessel mimic (BVM) systems developed in this thesis and their feasibility for the initial testing of newly emerging intravascular technologies has been demonstrated. There are limitations that exist with these designs, however, and the focus of this section is to discuss the limitations of the current models and future improvements that can be implemented to improve their design.

One design criteria that was not taken into consideration during the Ideal BVM development was the accommodation of physiological pressure and flow. The current BVM system setup uses low flow rates and low pressures. The performance of the Ideal BVM system has not been tested under high pressure, however, the thin compliant chamber walls may lead to failure. Under high pressure the walls may bellow, due to their flexibility, creating back pressure within the system. The ability of the lid to remain tightly sealed may also be compromised. Therefore, to improve the Ideal BVM system design it may be advantageous to use a container with more rigid walls that do not deform under high pressure. A stronger latching system may also be needed to prevent leakage from the lid.

Although stent delivery and deployment were not performed in the Ideal BVM system designed in this thesis, the procedure remains identical to the original BVM system. Stent delivery and deployment is performed by inserting a balloon catheter mounted with a coronary stent into the bioreactor system through the luminal inlet. Flow is temporarily stopped during the deployment procedure and requires the luminal inlet to be disconnected from the bioreactor system. It would be advantageous if stent delivery

and deployment could be performed without disconnecting the BVM system and stopping flow. One way that this could be accomplished is by attaching a touhy borst adapter via a Y-connector to a section of tubing leading to the luminal inlet. A touhy borst adapter allows small diameter devices, such as balloon catheters, to be inserted through a small seal which prevents leakage at the insertion site. The addition of this feature could allow stent delivery and deployment to be performed without disrupting flow within the BVM system and represents a more realistic stenting procedure.

Conclusion

As discussed in detail throughout this thesis, the blood vessel mimic system has the potential to help researchers predict the response of human tissue to intravascular devices prior to animal and clinical testing. The BVM system represents a more realistic and predictive model that could provide a high-throughput system for assessment of cellular response to novel intravascular technologies in order to direct animal and clinical studies toward the most promising devices. The benefits to medical device manufacturers and more importantly to patients needing these devices would be immense, resulting in better products reaching patients in a more timely fashion and at lower costs. With intravascular technologies advancing at a rapid pace, the BVM system has the potential to one day redefine how intravascular devices, specifically coronary stents, are tested and developed.

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APPENDIX A: LIST OF ABBREVIATIONS

AAA – Abdominal Aortic Aneurysm
ACC – American College of Cardiology
AHA – American Heart Association
BMS – Bare Metal Stent
BVM – Blood Vessel Mimic
CABG – Coronary Artery Bypass Graft
CAD – Coronary Artery Disease
CHD – Coronary Heart Disease
CT – Computerized Tomography
DES – Drug Eluting Stent
EC – Endothelial Cells
ECG/EKG – Electrocardiogram
ECHO – Echocardiogram
EO – Ethylene Oxide
ePTFE – Expanded Polytetrafluoroethylene
EVAR – Endovascular Aortic Aneurysm Repair
HA – Hyaluronic Acid
HUASMC – Human Umbilical Artery Smooth Muscle Cells
ID – Inner Diameter
ISR – In-stent Restenosis
IVUS – Intravascular Ultrasound
MRI – Magnetic Resonance Imaging
OD – Outer Diameter
PBMA – Poly-n-butyl Methacrylate
PC – Phosphorylcholine
PCI – Percutaneous Coronary Intervention
PCL – Polycaprolactone
PDLA – Poly-D,L-lactic Acid
PET – Positron Emission Tomography
PEVA – Polyethylene-co-vinyl Acetate
PGA – Polyglycolic Acid
PLGA – Polylactic-co-glycolic Acid
PLLA – Poly-L-lactic Acid
P/N – Part Number
PTCA – Percutaneous Transluminal Coronary Angioplasty
SEM – Scanning Electron Microscope
SMC – Smooth Muscle Cells
TEBV – Tissue Engineered Blood Vessels
VSMC – Vascular Smooth Muscle Cells
W – Width

APPENDIX B: PART INFORMATION AND DRAWINGS

Component	Description	Company	Website	Part Number
Chamber	Polypropylene Leak-Proof Container, 5"x4"x2", 350 ml	Lock & Lock	www.locknlock.com.au	HPL806
Chamber	Polypropylene Leak-Proof Container, 8"x5"x2", 800 ml	Lock & Lock	www.locknlock.com.au	HPL816
Buna-N O-Ring	Soft Buna-N O-Ring AS568A Dash Number 006	McMaster-Carr	www.mcmaster.com	2418T112
Loctite 4011	Loctite Prism 4011 Medical Adhesive, 20 Gram Bottle	McMaster-Carr	www.mcmaster.com	1818A4
Female Luer Lug Panel Mount-1/8" Barb	Female Luer Lug Style Panel Mount 1/4-28 UNF to 200 Series Barb, 1/8" (3.2 mm) ID Tubing, White Nylon	Value Plastics	www.valueplastics.com	FTLB230-1
Lock Nut	1/4-28 UNF Panel Mount Lock Nut, White Nylon	Value Plastics	www.valueplastics.com	LNS-1
Male Luer Lock-1/8" Barb	Male Luer Integral Lock Ring to 200 Series Barb, 1/8" (3.2 mm) ID Tubing, White Nylon	Value Plastics	www.valueplastics.com	MTLL230-1
Female Luer Thread-1/8" Barb	Female Luer Thread Style to 200 Series Barb, 1/8" (3.2 mm) ID Tubing, White Nylon	Value Plastics	www.valueplastics.com	FTLL230-1
Female Luer Thread-3/32" Barb	Female Luer Thread Style to 200 Series Barb, 3/32" (2.25 mm) ID Tubing, White Nylon	Value Plastics	www.valueplastics.com	FTLL220-1
Male-Female Coupler	Male Luer Integral Lock Ring to Female Luer Thread Style Coupler, White Nylon	Value Plastics	www.valueplastics.com	LC34-1
Y-Connector-1/8" Barbs	Y-Connector with 200 Series Barbs, 1/8" (3.2 mm) ID Tubing, White Nylon	Value Plastics	www.valueplastics.com	Y230-1
1-Way Stopcock	One-Way Stopcock, Capped Male Luer Lock to Capped Female Luer Thread Style, Polycarbonate Body w/ Polyethylene Handle	Value Plastics	www.valueplastics.com	VP455980

3-Way Stopcock	Three-Way Stopcock, 2 Capped Female Luer Thread Style & Capped Male Luer Lock, Polycarbonate Body w/ Polyethylene Handle	Value Plastics	www.valueplastics.com	VPB1000079N
Male Plug	Male Luer Integral Lock Ring Plug, Closed at Grip, White Nylon	Value Plastics	www.valueplastics.com	LP4-1
Male-to-Male Rotating Luer	Male-to-Male Luer Connector with One Rotating Collar, ABS	Qosina	www.qosina.com	17664
50 ml Tube	BD Falcon™ 50 ml Conical-Bottom Polypropylene Tube	BD Biosciences	www.bdbiosciences.com	352098
Silicone Tubing	Tygon® Silicone Tubing 1/8" ID x 1/4" OD	Cole-Parmer	www.coleparmer.com	WU-95702-06

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We Provide the best solution for food storage

Lock & Lock Regular

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- Square Container
- Divider Container
- Tall Container
- Long Container
- Round Container
- Rectangular Container

Lock & Lock Sets

- Lock & Lock Sets

NEW PRODUCTS

- New Products

Information on Regular Products

Use & Care

Rectangular Container

HPL806

- Color :Semitransparent
- Size :135 x 102 x 52mm
- Material:Polypropylene (P.P)
- Volume :350ml / 11OZ / 0.3QT

Feature

Size : 135 x 102 x 52mm / 72PCS / 0.056CBM / CTN

Composed of : 1 Container, 1 Lid Total : 2P

Square containers lock tightly to keep refrigerator odor out and keep food fresh. Good for storing various sauces, leftovers, canned goods, fruits, and vegetables. Refrigerator, freezer and microwave safe. Useful for other range of purposes as well.

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[Use & Care](#)

Rectangular Container

HPL816

- Color :Semitransparent
- Size :205 X 134 X 52mm
- Material:Polypropylene (P.P)
- Volume 800ml / 27OZ / 0.85QT

Feature

Size : 205 X 134 X 52mm / 60PCS / 0.095CBM / CTN

Composed of : 1 Container, 1 Lid Total : 2P

Square containers lock tightly to keep refrigerator odor out and keep food fresh. Good for storing various sauces, leftovers, canned goods, fruits, and vegetables. Refrigerator, freezer and microwave safe. Useful for other range of purposes as well.

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O-Rings, Cordstock, and Accessories
Part Number: 2418T112	\$10.00 per Pack of 150
	AS568A Dash Number	006
	Type	O-Ring
	O-Ring Type	Standard
	Cross Section Shape	Round
	Width	1/16"
	Actual Width	.070"
	Inside Diameter	1/8"
	Actual Inside Diameter	.114"
	Outside Diameter	1/4"
	Actual Outside Diameter	.254"
	Material	Buna-N
	Buna-N Type	Standard
	Durometer	Medium-Soft
	Durometer Shore	Shore A: 50
	Temperature Range	-40° to +250°F
	Color	Black
	Specifications Met	American Society for Testing and Materials (ASTM), Society of Automotive Engineers (SAE)
	ASTM Specification	ASTM D2000
	SAE Specification	SAE J200

Loctite® Adhesives and Accessories

Part Number:	1818A4	\$23.40 Each
Loctite® Brand	Prism®	
Loctite® Product Number	4011	
Loctite® Item Number	18680	
Package Size	.7 oz. (20 gram) bottle	
Color	Translucent

FOR REFERENCE ONLY

FOR REFERENCE ONLY

REVISIONS		
REV.	DESCRIPTION	DATE
A	ECO-0029	08-11-00
B	CO 013 Drafting Update	05-04-05 Rick C.

REVISIONS		
REV.	DESCRIPTION	DATE
A	ECO-0029	08-11-00
B	CO 013 Drafting Update	05-04-05 Rick C.

SECTION B-B

[illegible]

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NOTE: FOR MORE DETAILS SEE YOUR V.P. CUSTOMER SERVICE REP.

REVISIONS			
REV.	DESCRIPTION	DATE	APPROVED
A	INITIAL RELEASE	01-27-98	
B	CO 013 Drafting Update	05-02-05	Rick C.

Material Specific Tolerances								
Dimension	Acetal	Nylon	PP	ABS	PC	PVC	Polysulfone	J/A
A								
B								
C								
D								
E								
F								

VALUE PLASTICS, INC.			
USERS COMPANY SPECIFIED DIMENSIONS ARE IN INCHES	FRAC TIONS	DECIMALS	ANGLES
	1/16	.0625	1/2°
	1/8	.125	1/4°
	3/16	.1875	3/8°
	1/4	.25	1/2°
	5/16	.3125	5/8°
	3/8	.375	3/4°
	7/16	.4375	7/8°
	1/2	.5	1°
	9/16	.5625	1 1/8°
	5/8	.625	1 1/4°
	11/16	.6875	1 3/8°
	3/4	.75	1 1/2°
	13/16	.8125	1 5/8°
	7/8	.875	1 3/4°
	15/16	.9375	1 7/8°
	1	1.0	2°
	1 1/16	1.0625	2 1/8°
	1 1/8	1.125	2 1/4°
	1 1/4	1.25	2 1/2°
	1 1/2	1.5	3°
	1 5/8	1.625	3 1/8°
	1 3/4	1.75	3 1/4°
	1 7/8	1.875	3 3/8°
	2	2.0	3 1/2°
	2 1/16	2.0625	3 5/8°
	2 1/8	2.125	3 3/4°
	2 1/4	2.25	3 3/2°
	2 1/2	2.5	4°
	2 5/8	2.625	4 1/8°
	2 3/4	2.75	4 1/4°
	2 7/8	2.875	4 1/2°
	3	3.0	4 1/2°
	3 1/16	3.0625	4 3/8°
	3 1/8	3.125	4 3/4°
	3 1/4	3.25	4 3/2°
	3 1/2	3.5	5°
	3 5/8	3.625	5 1/8°
	3 3/4	3.75	5 1/4°
	3 7/8	3.875	5 1/2°
	4	4.0	5 1/2°
	4 1/16	4.0625	5 3/8°
	4 1/8	4.125	5 3/4°
	4 1/4	4.25	5 3/2°
	4 1/2	4.5	6°
	4 5/8	4.625	6 1/8°
	4 3/4	4.75	6 1/4°
	4 7/8	4.875	6 1/2°
	5	5.0	6 1/2°
	5 1/16	5.0625	6 3/8°
	5 1/8	5.125	6 3/4°
	5 1/4	5.25	6 3/2°
	5 1/2	5.5	7°
	5 5/8	5.625	7 1/8°
	5 3/4	5.75	7 1/4°
	5 7/8	5.875	7 1/2°
	6	6.0	7 1/2°
	6 1/16	6.0625	7 3/8°
	6 1/8	6.125	7 3/4°
	6 1/4	6.25	7 3/2°
	6 1/2	6.5	8°
	6 5/8	6.625	8 1/8°
	6 3/4	6.75	8 1/4°
	6 7/8	6.875	8 1/2°
	7	7.0	8 1/2°
	7 1/16	7.0625	8 3/8°
	7 1/8	7.125	8 3/4°
	7 1/4	7.25	8 3/2°
	7 1/2	7.5	9°
	7 5/8	7.625	9 1/8°
	7 3/4	7.75	9 1/4°
	7 7/8	7.875	9 1/2°
	8	8.0	9 1/2°
	8 1/16	8.0625	9 3/8°
	8 1/8	8.125	9 3/4°
	8 1/4	8.25	9 3/2°
	8 1/2	8.5	10°
	8 5/8	8.625	10 1/8°
	8 3/4	8.75	10 1/4°
	8 7/8	8.875	10 1/2°
	9	9.0	10 1/2°
	9 1/16	9.0625	10 3/8°
	9 1/8	9.125	10 3/4°
	9 1/4	9.25	10 3/2°
	9 1/2	9.5	11°
	9 5/8	9.625	11 1/8°
	9 3/4	9.75	11 1/4°
	9 7/8	9.875	11 1/2°
	10	10.0	11 1/2°
	10 1/16	10.0625	11 3/8°
	10 1/8	10.125	11 3/4°
	10 1/4	10.25	11 3/2°
	10 1/2	10.5	12°
	10 5/8	10.625	12 1/8°
	10 3/4	10.75	12 1/4°
	10 7/8	10.875	12 1/2°
	11	11.0	12 1/2°
	11 1/16	11.0625	12 3/8°
	11 1/8	11.125	12 3/4°
	11 1/4	11.25	12 3/2°
	11 1/2	11.5	13°
	11 5/8	11.625	13 1/8°
	11 3/4	11.75	13 1/4°
	11 7/8	11.875	13 1/2°
	12	12.0	13 1/2°
	12 1/16	12.0625	13 3/8°
	12 1/8	12.125	13 3/4°
	12 1/4	12.25	13 3/2°
	12 1/2	12.5	14°
	12 5/8	12.625	14 1/8°
	12 3/4	12.75	14 1/4°
	12 7/8	12.875	14 1/2°
	13	13.0	14 1/2°
	13 1/16	13.0625	14 3/8°
	13 1/8	13.125	14 3/4°
	13 1/4	13.25	14 3/2°
	13 1/2	13.5	15°
	13 5/8	13.625	15 1/8°
	13 3/4	13.75	15 1/4°
	13 7/8	13.875	15 1/2°
	14	14.0	15 1/2°
	14 1/16	14.0625	15 3/8°
	14 1/8	14.125	15 3/4°
	14 1/4	14.25	15 3/2°
	14 1/2	14.5	16°
	14 5/8	14.625	16 1/8°
	14 3/4	14.75	16 1/4°
	14 7/8	14.875	16 1/2°
	15	15.0	16 1/2°
	15 1/16	15.0625	16 3/8°
	15 1/8	15.125	16 3/4°
	15 1/4	15.25	16 3/2°
	15 1/2	15.5	17°
	15 5/8	15.625	17 1/8°
	15 3/4	15.75	17 1/4°
	15 7/8	15.875	17 1/2°
	16	16.0	17 1/2°
	16 1/16	16.0625	17 3/8°
	16 1/8	16.125	17 3/4°
	16 1/4	16.25	17 3/2°
	16 1/2	16.5	18°
	16 5/8	16.625	18 1/8°
	16 3/4	16.75	18 1/4°
	16 7/8	16.875	18 1/2°
	17	17.0	18 1/2°
	17 1/16	17.0625	18 3/8°
	17 1/8	17.125	18 3/4°
	17 1/4	17.25	18 3/2°
	17 1/2	17.5	19°
	17 5/8	17.625	19 1/8°
	17 3/4	17.75	19 1/4°
	17 7/8	17.875	19 1/2°
	18	18.0	19 1/2°
	18 1/16	18.0625	19 3/8°
	18 1/8	18.125	19 3/4°
	18 1/4	18.25	19 3/2°
	18 1/2	18.5	20°
	18 5/8	18.625	20 1/8°
	18 3/4	18.75	20 1/4°
	18 7/8	18.875	20 1/2°
	19	19.0	20 1/2°
	19 1/16	19.0625	20 3/8°
	19 1/8	19.125	20 3/4°
	19 1/4	19.25	20 3/2°
	19 1/2	19.5	21°
	19 5/8	19.625	21 1/8°
	19 3/4	19.75	21 1/4°
	19 7/8	19.875	21 1/2°
	20	20.0	21 1/2°
	20 1/16	20.0625	21 3/8°
	20 1/8	20.125	21 3/4°
	20 1/4	20.25	21 3/2°
	20 1/2	20.5	22°
	20 5/8	20.625	22 1/8°
	20 3/4	20.75	22 1/4°
	20 7/8	20.875	22 1/2°
	21	21.0	22 1/2°
	21 1/16	21.0625	22 3/8°
	21 1/8	21.125	22 3/4°
	21 1/4	21.25	22 3/2°
	21 1/2	21.5	23°
	21 5/8	21.625	23 1/8°
	21 3/4	21.75	23 1/4°
	21 7/8	21.875	23 1/2°
	22	22.0	23 1/2°
	22 1/16	22.0625	23 3/8°
	22 1/8	22.125	23 3/4°
	22 1/4	22.25	23 3/2°
	22 1/2	22.5	24°
	22 5/8	22.625	24 1/8°
	22 3/4	22.75	24 1/4°
	22 7/8	22.875	24 1/2°
	23	23.0	24 1/2°
	23 1/16	23.0625	24 3/8°
	23 1/8	23.125	24 3/4°
	23 1/4	23.25	24 3/2°
	23 1/2	23.5	25°
	23 5/8	23.625	25 1/8°
	23 3/4	23.75	25 1/4°
	23 7/8	23.875	25 1/2°
	24	24.0	25 1/2°
	24 1/16	24.0625	25 3/8°
	24 1/8	24.125	25 3/4°
	24 1/4	24.25	25 3/2°
	24 1/2	24.5	26°
	24 5/8	24.625	26 1/8°
	24 3/4	24.75	26 1/4°
	24 7/8	24.875	26 1/2°
	25	25.0	26 1/2°
	25 1/16	25.0625	26 3/8°
	25 1/8	25.125	26 3/4°
	25 1/4	25.25	26 3/2°
	25 1/2	25.5	27°
	25 5/8	25.625	27 1/8°
	25 3/4	25.75	27 1/4°
	25 7/8	25.875	27 1/2°
	26	26.0	27 1/2°
	26 1/16	26.0625	27 3/8°
	26 1/8	26.125	27 3/4°
	26 1/4	26.25	27 3/2°
	26 1/2	26.5	28°
	26 5/8	26.625	28 1/8°
	26 3/4	26.75	28 1/4°
	26 7/8	26.875	28 1/2°
	27	27.0	28 1/2°
	27 1/16	27.0625	28 3/8°
	27 1/8	27.125	28 3/4°
	27 1/4	27.25	28 3/2°
	27 1/2	27.5	29°
	27 5/8	27.625	29 1/8°
	27 3/4	27.75	29 1/4°
	27 7/8	27.875	29 1/2°
	28	28.0	29 1/2°
	28 1/16	28.0625	29 3/8°
	28 1/8	28.125	29 3/4°
	28 1/4	28.25	29 3/2°
	28 1/2	28.5	30°
	28 5/8	28.625	30 1/8°
	28 3/4	28.75	30 1/4°
	28 7/8	28.875	30 1/2°
	29	29.0	30 1/2°
	29 1/16	29.0625	30 3/8°
	29 1/8	29.125	30 3/4°
	29 1/4	29.25	30 3/2°
	29 1/2	29.5	31°
	29 5/8	29.625	31 1/8°
	29 3/4	29.75	31 1/4°
	29 7/8	29.875	31 1/2°
	30	30.0	31 1/2°
	30 1/16	30.0625	31 3/8°
	30 1/8	30.125	31 3/4°
	30 1/4	30.25	31 3/2°
	30 1/2	30.5	32°
	30 5/8	30.625	32 1/8°
	30 3/4	30.75	32 1/4°
	30 7/8	30.875	32 1/2°
	31	31.0	32 1/2°
	31 1/16	31.0625	32 3/8°
	31 1/8	31.125	32 3/4°
	31 1/4	31.25	32 3/2°
	31 1/2	31.5	33°
	31 5/8	31.625	33 1/8°
	31 3/4	31.75	33 1/4°
	31 7/8	31.875	33 1/2°
	32	32.0	33 1/2°
	32 1/16	32.0625	33 3/8°
	32 1/8	32.125	33 3/4°
	32 1/4	32.25	33 3/2°
	32 1/2	32.5	34°
	32 5/8	32.625	34 1/8°
	32 3/4	32.75	34 1/4°
	32 7/8	32.875	34 1/2°
	33	33.0	34 1/2°
	33 1/16	33.0625	34 3/8°
	33 1/8	33.125	34 3/4°
	33 1/4	33.25	34 3/2°
	33 1/2	33.5	35°
	33 5/8	33.625	35 1/8°
	33 3/4	33.75	35 1/4°
	33 7/8	33.875	35 1/2°
	34	34.0	35 1/2°
	34 1/16	34.0625	35 3/8°
	34 1/8	34.125	35 3/4°
	34 1/4	34.25	35 3/2°
	34 1/2	34.5	36°
	34 5/8	34.625	36 1/8°
	34 3/4	34.75	36 1/4°
	34 7/8	34.875	36 1/2°
	35	35.0	36 1/2°
	35 1/16	35.0625	36 3/8°
	35 1/8	35.125	36 3/4°
	35 1/4	35.25	36 3/2°
	35 1/2	35.5	37°
	35 5/8	35.625	37 1/8°
	35 3/4	35.75	37 1/4°
	35 7/8	35.875	37 1/2°
	36	36.0	37 1/2°
	36 1/16	36.0625	37 3/8°
	36 1/8	36.125	37 3/4°
	36 1/4	36.25	37 3/2°
	36 1/2	36.5	38°
	36 5/8	36.625	38 1/8°
	36 3/4	36.75	38 1/4°
	36 7/8	36.875	38 1/2°
	37		

NOTE: FOR MORE DETAILS SEE YOUR V.P. CUSTOMER SERVICE REP.

REVISIONS			
REV.	DESCRIPTION	DATE	APPROVED
A	INITIAL RELEASE	11-21-02	
B	CO 013 Drafting Update	05-02-05	Rick C.

SECTION B - B

Dimension	Material Specific Tolerances					
	Acetal	Nylon	PP	ABS	PC	PVC Polysulfone J/A
A						
B						
C						
D						
E						
F						

VALUE PLASTICS, INC. 2004 VALUE PLASTICS, INC.		PART NUMBER: FTLL220 DESCRIPTION: Female Luer Thread Style to 200 Series Barb, 3/32" (2.25 mm) ID Tubing
FRANK LOMBARDI 4-18-05 RICK CALHUN	NTS 1005489	SHEET: 1 OF 1

FOR REFERENCE ONLY

FOR REFERENCE ONLY

REV. NO.			REVISED		DATE	APPROVED
REV.	DESCRIPTION	INITIALS	DATE	DATE	DATE	DATE
A.	INITIAL RELEASE				12-27-02	
B	CO 013 Drafting Update				05-02-05	Rick C.
C	ECO 065-Changed Nylon and PF Nominal to 0.184"				10-30-07	Rick C.

[illegible]

FOR REFERENCE ONLY

FOR INFORMATION ATTN

B. Braun Medical Inc. Bethlehem, PA 18018	
D-300 STOPCOCK ASSY.	
PART OR PRODUCT NO.:	B1000079
APPROVED BY:	
DATE	7-20-92 RM
SCALE	2:1
DWG. NO.	CP-419
REV. NO.	

FOR INFORMATION ATT

REVISIONS			
REV.	DESCRIPTION	DATE	APPROVED
A	INITIAL RELEASE	02-05-98	
B	CO 027 Drafting Update	01-17-06	Blck C.

SECTION B-B

UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES DIMENSIONS ARE IN MILLIMETERS	UNLESS OTHERWISE SPECIFIED DIMENSIONS ARE IN INCHES DIMENSIONS ARE IN MILLIMETERS	VALVE PLASTICS, INC.
DRAWING NO. 08-15-05 Frank Lombardi DATE 08-15-05	DRAWING NO. 08-15-05 Frank Lombardi DATE 08-15-05	PARTS LF4 NUMBER: Male Luer Integral Lock Description: Ring Plug, Closed at Grip
FINISHES 1. 1.001 2. 1.001 3. 1.001 4. 1.001 5. 1.001 6. 1.001 7. 1.001 8. 1.001 9. 1.001 10. 1.001 11. 1.001 12. 1.001 13. 1.001 14. 1.001 15. 1.001 16. 1.001 17. 1.001 18. 1.001 19. 1.001 20. 1.001 21. 1.001 22. 1.001 23. 1.001 24. 1.001 25. 1.001 26. 1.001 27. 1.001 28. 1.001 29. 1.001 30. 1.001 31. 1.001 32. 1.001 33. 1.001 34. 1.001 35. 1.001 36. 1.001 37. 1.001 38. 1.001 39. 1.001 40. 1.001 41. 1.001 42. 1.001 43. 1.001 44. 1.001 45. 1.001 46. 1.001 47. 1.001 48. 1.001 49. 1.001 50. 1.001 51. 1.001 52. 1.001 53. 1.001 54. 1.001 55. 1.001 56. 1.001 57. 1.001 58. 1.001 59. 1.001 60. 1.001 61. 1.001 62. 1.001 63. 1.001 64. 1.001 65. 1.001 66. 1.001 67. 1.001 68. 1.001 69. 1.001 70. 1.001 71. 1.001 72. 1.001 73. 1.001 74. 1.001 75. 1.001 76. 1.001 77. 1.001 78. 1.001 79. 1.001 80. 1.001 81. 1.001 82. 1.001 83. 1.001 84. 1.001 85. 1.001 86. 1.001 87. 1.001 88. 1.001 89. 1.001 90. 1.001 91. 1.001 92. 1.001 93. 1.001 94. 1.001 95. 1.001 96. 1.001 97. 1.001 98. 1.001 99. 1.001 100. 1.001	FINISHES 1. 1.001 2. 1.001 3. 1.001 4. 1.001 5. 1.001 6. 1.001 7. 1.001 8. 1.001 9. 1.001 10. 1.001 11. 1.001 12. 1.001 13. 1.001 14. 1.001 15. 1.001 16. 1.001 17. 1.001 18. 1.001 19. 1.001 20. 1.001 21. 1.001 22. 1.001 23. 1.001 24. 1.001 25. 1.001 26. 1.001 27. 1.001 28. 1.001 29. 1.001 30. 1.001 31. 1.001 32. 1.001 33. 1.001 34. 1.001 35. 1.001 36. 1.001 37. 1.001 38. 1.001 39. 1.001 40. 1.001 41. 1.001 42. 1.001 43. 1.001 44. 1.001 45. 1.001 46. 1.001 47. 1.001 48. 1.001 49. 1.001 50. 1.001 51. 1.001 52. 1.001 53. 1.001 54. 1.001 55. 1.001 56. 1.001 57. 1.001 58. 1.001 59. 1.001 60. 1.001 61. 1.001 62. 1.001 63. 1.001 64. 1.001 65. 1.001 66. 1.001 67. 1.001 68. 1.001 69. 1.001 70. 1.001 71. 1.001 72. 1.001 73. 1.001 74. 1.001 75. 1.001 76. 1.001 77. 1.001 78. 1.001 79. 1.001 80. 1.001 81. 1.001 82. 1.001 83. 1.001 84. 1.001 85. 1.001 86. 1.001 87. 1.001 88. 1.001 89. 1.001 90. 1.001 91. 1.001 92. 1.001 93. 1.001 94. 1.001 95. 1.001 96. 1.001 97. 1.001 98. 1.001 99. 1.001 100. 1.001	TITLE: SCALE: PARTS NTS DRAWING NUMBER: 100075.27 REV. B SHEET OF 1 © 2005 VALVE PLASTICS, INC.

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Part Number: 17664 **ADD TO SAMPLE CART**

Description: Male to Male Luer Connector with One Rotating Collar

Material: [ABS](#) **ALTERNATE PRODUCTS**

Category: [Luers, Male to Male](#)

Packaging: Bulk

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17664

This component can be viewed in the [Qosina 2008 print catalog](#) on page 22.

Dimensions are for reference only

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Catalog Number 352098
Brand BD Falcon™
Name Conical Tubes
Size 50 ml
Regulatory Status Class I Medical
Catalog Number 352098
List Price \$269.00 [Pricing Disclaimer](#)

Labware details
Material : tube - Polypropylene
Sterile : Yes
Quantity Pack : 25 **type :** rack
Quantity Case : 500
Packaging : 25/rack, 500/case

Shape : Conical
Dim nominal : 30x115 mm
Cap : Flat-top screw cap
Writing patch: Yes
Rcf rating : 9400
Vol nominal : 50.0 ml

Documents
[TDS \(PDF\)](#)

Description
50 ml high-clarity polypropylene conical centrifuge tube, 9400 RCF rating. Sterile. 25/rack, 500/case.

Images

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Tygon[®] Silicone Tubing - Product Detail

WU-95702-06 (7 of 20) [\[Previous\]](#) [\[Next\]](#)

Tygon[®] Silicone Tubing

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WU-95702-06
Tygon[®] silicone tubing, 1/8"ID x 1/4"OD, 50 feet/package (package)
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Formulation: TYGON 3350 silicone, platinum cured.

Applications: Pharmaceutical, cosmetic, medical, industrial, and auto-analysis applications.

Characteristics: Ultra-smooth inner surface reduces particle entrapment and minimizes bacterial growth. Good for mild to medium concentration bases, salts, and alcohols; is odorless, tasteless, and non-toxic. Transparent.

Certification: Elastomer is FDA-compliant (21 CFR 177.2600); meets USP 23 Class VI requirements, and NSF criteria. Tubing is manufactured according to GMP.

Temperature range: -112 to 392°F (-80 to 200°C)

Sterilize: With ethylene oxide, gamma irradiation, or autoclave for 30 min, 15 psi.

Specifications

Material	Tygon [®] Silicone
ID	1/8" (3.2 mm)
OD	1/4" (6.4 mm)
Wall thickness	1/16" (1.6 mm)
Max psi	9 psi at 73°F
Certifications	FDA, USP, NSF, GMP

Technical Appendix

- Technical Library** (roll over link for article summary)
 - [Cole-Parmer's Bioconnect Program](#)
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APPENDIX C: IDEAL BVM SYSTEM ASSEMBLY

Component	Company	Part Number	Quantity
Leak-Proof Chamber	Lock & Lock	HPL806	1 EA
Buna-N O-Ring	McMaster-Carr	2418T112	6 EA
Loctite 4011 Adhesive	McMaster-Carr	1818A4	3 drops
Female Luer Lug Panel Mount-1/8" Barb	Value Plastics	FTLB230-1	3 EA
Lock Nut	Value Plastics	LNS-1	3 EA
Male Luer Lock-1/8" Barb	Value Plastics	MTLL230-1	2 EA
Female Luer Thread-1/8" Barb	Value Plastics	FTLL230-1	2 EA
Y-Connector-1/8" Barbs	Value Plastics	Y230-1	1 EA
Male-Female Coupler	Value Plastics	LC34-1	2 EA
1-Way Stopcock	Value Plastics	VP455980	2 EA
3-Way Stopcock	Value Plastics	VPB1000079N	1 EA
Male-to-Male Rotating Luer Lock	Qosina	17664	2 EA
Tygon® Silicone Tubing, 1/8" ID	Cole-Parmer	WU-95702-06	11 inches

1. Drill three 15/64" holes into the sides of the leak-proof chamber (Lock & Lock P/N HPL806).

- a. Drill the luminal inlet hole on the left face of the chamber 1 cm down from the top centered notch and 1 cm to the right.

- b. Drill the luminal outlet hole on the right face of the chamber 1 cm down from the top centered notch and 1 cm to the left.
- c. Drill the extra luminal outlet hole on the right face of the chamber 1 cm down from the top centered notch and 2 cm to the right.

- 2. Using a razor blade, remove any plastic debris that resulted from the use of the drill from the edges of the holes.

- 3. Slide a Buna-N o-ring (McMaster-Carr P/N 2418T112) onto a female luer lug panel mount-1/8" barb (Value Plastics P/N FTLB230-1).

4. Thread the female luer lug panel mount with the Buna-N o-ring through the drilled luminal inlet hole with the barbed portion exiting the side of the chamber.

5. Slide a second Buna-N o-ring (McMaster-Carr P/N 2418T112) onto the barbed end of the female luer lug panel mount.

6. Tightly screw a lock nut (Value Plastics P/N LNS-1) onto the female luer lug panel mount, pressing the o-rings against the inside and outside walls of the chamber.

7. Once securely tightened, place a drop of Loctite 4011 adhesive (McMaster-Carr P/N 1818A4) between the lock nut and the threaded portion of the female luer lug panel mount.

8. Repeat steps 3-7 for assembly of the luminal outlet and extra luminal outlet holes.

9. Attach a 2" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed fitting of the luminal inlet.

10. Attach a female luer thread-1/8" ID barb (Value Plastics P/N FTLL230-1) to the open end of the silicone tubing attached to the luminal inlet.

11. Secure the male luer lock end of a 3-way stopcock (Value Plastics P/N VPB1000079N) to the female luer thread attached to the silicone tubing.

12. Attach a 5" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed fitting of the extra luminal outlet.

13. Attach a 1" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed fitting of the luminal outlet.

14. Attach a male luer lock-1/8" ID barb (Value Plastics P/N MTLL230-1) to the open end of the silicone tubing attached to the luminal outlet.

15. Secure the female luer thread end of a 1-way stopcock (Value Plastics P/N VP455980) to the male luer lock attached to the silicone tubing.

16. Attach a female luer thread-1/8" ID barb (Value Plastics P/N FTLL230-1) to the male luer lock end of the 1-way stopcock.

17. Attach a 2" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed fitting of the female luer thread attached to the 1-way stopcock.

18. Insert the open ends of the silicone tubing attached to the luminal outlet and extra luminal outlet to a Y-connector with 1/8" ID barbs (Value Plastics P/N Y230-1).

19. Attach a 1" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed fitting of the Y-connector.

20. Attach a male luer lock-1/8" ID barb (Value Plastics P/N MTLL230-1) to the open end of the silicone tubing attached to the Y-connector.

21. Secure the female luer thread end of a 1-way stopcock (Value Plastics P/N VP455980) to the male luer lock attached to the silicone tubing.

22. Place the lid with built in silicone o-ring on the assembled Ideal BVM chamber and close the four snapping latches.

APPENDIX D: TWO-PORT MEDIA RESERVOIR ASSEMBLY

Component	Company	Part Number	Quantity
BD Falcon™ 50 ml Tube	BD Biosciences	352098	1 EA
Buna-N O-Ring	McMaster-Carr	2418T112	4 EA
Loctite 4011 Adhesive	McMaster-Carr	1818A4	2 drops
Female Luer Lug Panel Mount-1/8" Barb	Value Plastics	FTLB230-1	2 EA
Lock Nut	Value Plastics	LNS-1	2 EA
Male Luer Lock-1/8" Barb	Value Plastics	MTLL230-1	2 EA
Female Luer Thread-1/8" Barb	Value Plastics	FTLL230-1	1 EA
Tygon® Silicone Tubing, 1/8" ID	Cole-Parmer	WU-95702-06	21 inches

1. Drill two 15/64" holes into the lid of a BD Falcon™ 50 ml tube (BD Biosciences P/N 352098).

2. Using a razor blade, remove any plastic debris that resulted from the use of the drill from the edges of the holes.

3. Slide a Buna-N o-ring (McMaster-Carr P/N 2418T112) onto a female luer lug panel mount-1/8" barb (Value Plastics P/N FTLB230-1).

4. Thread the female luer lug panel mount with the Buna-N o-ring through one of the drilled holes with the barbed portion exiting the top of the lid.

5. Slide a second Buna-N o-ring (McMaster-Carr P/N 2418T112) onto the barbed end of the female luer lug panel mount.

6. Tightly screw a lock nut (Value Plastics P/N LNS-1) onto the female luer lug panel mount, pressing the o-rings against the top and bottom of the lid.

7. Once securely tightened, place a drop of Loctite 4011 adhesive (McMaster-Carr P/N 1818A4) between the lock nut and the threaded portion of the female luer lug panel mount.

8. Repeat steps 3-7 for assembly of the second hole.

9. Attach a 1" piece of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to a male luer lock-1/8" ID barb (Value Plastics P/N MTLL230-1).

10. Secure the male luer lock fitting of the silicone tubing assembly to one of the female luer lug panel mounts exiting the bottom of the lid.

11. Attach two 10" pieces of Tygon® silicone tubing, 1/8" ID (Cole-Parmer P/N WU-95702-06) to the barbed ends of the female luer lug panel mounts exiting the top of the lid.

12. Attach a male luer lock-1/8" ID barb (Value Plastics P/N MTLL230-1) to the open end of the silicone tubing attached to the female luer lug panel mount with the 1" silicone tubing assembly.

13. Attach a female luer thread-1/8" ID barb (Value Plastics P/N FTLL230-1) to the open end of the silicone tubing attached to the other female luer lug panel mount.

14. Screw the bottom section of the BD Falcon™ 50 ml tube to the assembled two-port media reservoir lid.

APPENDIX E: SCAFFOLD LENGTH VS NUMBER OF COUPLERS USED

Illustration	# of Couplers	Scaffold Length
0	5.5 cm
1	4.5 cm
2	3.5 cm

APPENDIX F: BVM SYSTEM USER FEEDBACK SURVEY

Name: _____

Date: _____

1. Overall Usability	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

2. Setup	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

3. Scaffold Assembly	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

4. Scaffold Insertion	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

5. Media Injection	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

6. Seal-ability	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

7. Aseptic Use	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

8. Bioreactor Assembly	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

9. Control of Flow	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

10. Adjustability	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

11. Components and Fittings	Awkward				Convenient
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

12. Component Configuration	Poor				Excellent
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

BVM SYSTEM USER FEEDBACK SURVEY

Name: Dimitri Delagrammaticas

Date: 07/01/08

1. Overall Usability	Difficult					Easy
Original BVM	1	2	<u>3</u>	4	5	
Ideal BVM	1	2	3	<u>4</u>	5	

Comments:

still need a non-sterile partner but fittings and lid make overall use
easier

2. Setup	Difficult					Easy
Original BVM	1	2	<u>3</u>	4	5	
Ideal BVM	1	2	3	4	<u>5</u>	

Comments:

separation of lid from chamber makes setup easier; male and female
fittings ensure correct setup; no air bubbles; no tools needed

3. Scaffold Assembly	Difficult					Easy
Original BVM	1	2	3	<u>4</u>	5	
Ideal BVM	1	2	3	<u>4</u>	5	

Comments:

no difference between old and new BVM; scaffold setup is easy

4. Scaffold Insertion	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

lid does not have to be held freeing up an extra hand for use; rotating
luer lock fittings make insertion very easy

5. Media Injection	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

media injection is the same for both systems through the three-way
stopcock; air bubbles are easier to eliminate from new chamber but
new chamber requires more media

6. Seal-ability	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

built-in o-ring seal is nice; lid is easy to snap to chamber and creates a strong
leak-proof seal; no tools needed when snapping on lid

7. Aseptic Use	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

separating the lid from the chamber improves aseptic use; lid can be
sterilized individually

8. Bioreactor Assembly	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

only one way to connect chamber to media reservoir; fast and easy
but new chamber is larger

9. Control of Flow	Difficult				Easy
Original BVM	1	2	3	4	5
Ideal BVM	1	2	3	4	5

Comments:

stopcocks are easier to use than side clamps and firmly block flow;
placement of one-way stopcocks makes control of flow easy

10. Adjustability	Difficult				Easy
Original BVM	1	2	3	4	<u>5</u>
Ideal BVM	1	2	3	<u>4</u>	5

Comments:

adjustability is limited to couplers; adjusting with couplers is easy and
provides consistent scaffold lengths

11. Components and Fittings	Awkward				Convenient
Original BVM	1	2	3	<u>4</u>	5
Ideal BVM	1	2	3	4	<u>5</u>

Comments:

elimination of UV glue improves component setup; components and
fittings are simple to use

12. Component Configuration	Poor				Excellent
Original BVM	1	2	3	<u>4</u>	5
Ideal BVM	1	2	3	4	<u>5</u>

Comments:

one-way connections to reservoir eliminates the confusion on which
end to connect when assembling the complete bioreactor system;
connection piece used in media reservoir sterilization is eliminated

APPENDIX G: REPRINT PERMISSIONS

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September 8, 2008

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