CHAPTER 1

INTRODUCTION TO PROSTHETIC HEART VALVES AND ITS COMPLICATIONS, NANOTECHNOLOGY AND ITS APPLICATION IN BIOLOGY

1.1 INTRODUCTION

1.2 ANATOMY OF HEART AND ITS FUNCTION

Heart is a pulsatile fist sized organ with an average weight of 250-350 grams and located in the thoracic cavity between right and left lung with thoracic spine behind, sternum in front and diaphragm beneath. It lies in the pericardium which is a fluid sac. It has three layered muscular wall, epicardium, myocardium and endocardium. Heart is highly reliable pump designed to work continuously throughout the life span and circulate to all parts of the body. It has four chambers and four walls, right atrium and left atrium are the upper chambers while right ventricle and left ventricle are the lower chambers separated by atrial and ventricular septum respectively. The upper chambers of the heart functions primarily as reservoirs and the ventricles functions as the pumping chambers.

The heart functions as a pump and circulates about five liters of blood through the vessels to all parts of the body. The right atrium and right ventricle controls pulmonary circulation while the left atrium and left ventricle controls systemic circulation. The deoxygenated blood received by the right atrium is transported to the right ventricle and to the pulmonary artery to lungs for oxygenation process. The oxygenated blood from the lungs
is received by the left atrium then to left ventricle and to the aorta for circulation. The septum between the auricles and ventricles prevents mixing of oxygenated and deoxygenated blood. The atrium and ventricles contract and relax in a coordinated manner to complete one cycle of circulation. The relaxation period is called diastole during which the heart fills with blood followed by contraction period called systole. This cyclic rhythmic process occurs about seventy two times per minute throughout the span of life.

1.2.1 Valves of the heart

The heart is composed of four valves which allow flow of blood in only one direction without causing any backflow of blood. The pathway for the flow of blood through the heart is determined by the valves in the heart. The valves are located at exit point of each chamber. The two atrio-ventricular valves are situated between the atria and ventricles. The bicuspid or the mitral valve opens from the left atrium to left ventricle while tricuspid valve opens from left atrium to the right ventricle. Pulmonary valve located between the right ventricle and pulmonary artery, opens from the arteries leaving the heart. Aortic valve open into the aorta from left ventricle and pulmonary valve open into the the pulmonary artery from right ventricle. The valves are leaflets or cusps made of thin but strong flaps of tissues. Mitral valve is composed only of two cusps while the other three valves have three cusps.

1.2.2 Heart Valve Diseases

Heart valve diseases have created a great impact resulting in one of the primary cause of cardiovascular morbidity and mortality worldwide (Thomas et al. 2003). It has been estimated that annually 275,000 valve replacements taking place globally (Rabkin et al. 2002). According to the World Health Organization, 17.5 million people are died because of cardiovascular diseases in 2005 representing virtually 30% of mortalities (Nkomo et al. 2006). The cause for the disease is mainly due to heart valve
failures which is estimated to triple from 290,000 in 2003 to an vast increase of about 850,000 by 2050 (Yacoub et al. 2005; Filova et al. 2009). The cause for the heart valve diseases is mainly due to the malfunctioning of the valves (Cooper et al. 1966; Bachetti et al. 2000). The conditions that can cause damage to the heart valves include birth defects, age-related changes (valve tissue degenerate with age), endocarditis infection, and calcification of the valve, rheumatic disease, heart muscle disease (cardiomyopathy), building of plaque inside the arteries (atherosclerosis) and other conditions (Butany et al. 2003; Bhuvaneshwar et al. 1991). The aforementioned conditions make the heart to work harder in order to pump the blood thereby affecting its ability.

1.2.3 Valve stenosis and valve incompetence

When the leaflets of the heart become thickened or rigid, the valve through which the blood passes from one chamber to another chamber gets narrowed leading to obstruction in the forward flow of blood which is called stenosis. When the flow of blood is obstructed, there is accumulation of blood in the chamber causing the heart to pump harder to push it to the next chamber. The second condition is called incompetence or valve regurgitation in which the valve does not close properly and there is a backflow of blood due to the ejection of blood. Both these complications cause a serious strain on the heart. When the valves become more narrow or leaky, there is devastating impact associated with these complications. Due to this fact, there is an increase in pressure behind the valve that was affected. The resulting back pressure will cause blood and fluid to build up in the lungs or lower parts of the body depending on the valve that was affected. If the narrowing or leakiness is severe with associated complications valve repair or replacement is an alternative option which has greatly improved the lives of many cases of severe heart valve diseases.
1.3 VALVE REPLACEMENTS

Valve replacement is the removal of diseased valve and a new valve is inserted in their position. In cases of damaging of the heart valve resulting in severe impediment, the diseased valves that is life threatening must be repaired or replaced. When normal function of the heart valve gets affected, drugs can be used to relieve the symptoms but that is not the remedy for complete or permanent cure or to reverse the disease (Michae et al. 2007; Blackstone and Kirklin 1985; Lamas and Eykyn 2000). Valve replacement surgery is recommended when damage to the valve is significant in causing a life threatening risk. During valve repair procedure; a ring will be sewn near the opening of the valve to tighten them. Other parts may be cut, separated, shortened or made stronger by artificial means to make the valve open and...
Bioprosthetic heart valves (BHVs) do not have long-term thrombogenicity problems due to their biocompatibility and improved physiological haemodynamics but have a shorter lifespan induced by the glutaraldehyde fixing process of the pericardium (Siddiqui et al. 2009; Edmunds et al. 1996; Jin et al. 1998). The bioprosthetic valve reproduces the central flow characteristics like that of native heart valve and is less likely to cause thrombosis than mechanical valves (Pibarot and Dumesnil 2009). For this reason, bioprosthetic valves have become the first choice for patients with a life expectancy less than 10-15 years (Cunanan et al. 2001). Bioprosthetic valves use biological materials namely vessel tissue from a donor or an
animal or the valve itself. The biological valves first appeared on the market in the 1970’s but continue to have been plagued with many problems with results of reduction in durability (Vesely 2003) in comparison to the mechanical valve options. The types of biological valve include stented porcine, stentless porcine xenografts, pericardial xenografts, autografts and cadaveric homografts. The stented porcine valves and bovine pericardium valves are the most widely implanted biological valves when compared to stentless grafts.

Xenogenic heart valves are mainly fabricated from bovine or porcine heart valves or from animal pericardial tissue. These types of valves differ on the construction type, nature and chemical fixation. They are chemically fixed using glutaraldehyde for heart valve replacement (Broom and Thomson 1979). The advantage of xenograft relies on the fact that they are similar to human valves and their availability makes them ideal biological valves in clinical applications. The bioprosthetic valves that are fabricated from bovine pericardial and porcine aortic valves showed excellent outcomes by reducing risk of thrombosis and improvement in the haemocompatibility (Konakci et al. 2005) without the need for anticoagulation treatment. Despite showing encouraging outcomes, these valves are prone to tissue degeneration affecting the durability of the valves. The factors that are responsible for valve structural deterioration are dependent on the morphological and chemical changes that are taking place during the process of fabrication (Butany and Leask 2001); these changes will have strong influence on the tissue and play a serious role in causing mechanical damage. The other issue which poses threat to the functionality of valve after implantation is the increase in hemodynamic shear stress. The durability of the valve also depends on various factors namely the nature of the tissue, process of chemical fixation, method adopted for preservation, patient’s age and medical
condition, donor and recipient tissue immunological reactions and the hemodynamic properties.

**Stented valves** are another kind of widely used bioprosthetic valves fabricated from tissue. These types of valves are mounted on metallic or stent made of plastic surrounded by sewing ring to its base structure. The sewing ring on the base will facilitate proper fixation of the graft implanted, but the ring of the valve reduces the opening area of the graft that was implanted. The differences in the valve opening and prosthesis ring shape are greatly associated with increased gradient that would lead to an increase in the stress (Ruel et al. 2004). These factors may attribute to the cardiac function as well as durability of the valve.

**Stentless valves** have been developed in the early 1990s to improve the haemodynamic characteristics and also to overcome the problem of patient prosthesis mismatch. These valves are sewn into the valve site directly either by adopting root method or sub-coronary technique. But surgical insertions of valve by both methods are accompanied with serious complications like bleeding or valve regurgitation. These types of valve are suitable only for aortic valve replacement and are not permitted for mitral and tricuspid valve replacements (Thomas et al. 2003).

The other type of biprosthetic valve is called **human allograft valves** which have several advantages than the existing valves. They are harvested from recipients of heart transplant or from the cadavers. The advantage of allograft over other types of biological valves is their infection resistant properties and excellent haemodynamic characteristics (Freeman and Otto 2005). They are mostly preferred in cases of severe endocarditis because of their perfect configuration to the valve annulus of the recipient. Factors that are strongly responsible for the homograft durability depends on the method of harvesting, protocol designed for preservation, ideal implantation technique
and the selection of the patients. Initially the homograft replacements were carried out using antibiotic sterilized and valves that are wet stored, but due to less durability they were replaced by allografts that are cryopreserved. Recent times the homografts are frozen in dimethyl sulfoxide and preserved at $-196^\circ$C. The viable cells present can be stored for years and will tend to retain the durability. The biggest advantage associated with this type of valve is that they are less frequently prone to infection and reduced complexity of valve replacement procedure than the standard replacements of heart valves. Several other issues concerned with the homograft include size, length and the longevity of the valve. The negative aspect of this type of valve is their incapable nature to grow collaterally with the growth of recipient body and hence prone to repeated replacement or re-operative procedures in paediatric patients (Butany et al. 2003).

Figure 1.3 Types of bioprosthetic heart valve (A) Allograft valve (B) Xenograft and (C) Homograft (Butany et al. 2003)

It is proposed that using **autologous pulmonary** valve, diseased aortic or a mitral valve can be reconstructed (Bechtel et al. 2006). In this type of replacement, the individual’s own pulmonary valve can be substituted with xenograft fixed glutaraldehyde or with a cryopreserved allograft. Due to low shear stress, the valves replaced will tend to degenerate in the pulmonary position. Like other bioprosthetic valves, the allografts also provide better
haemodynamic properties and eliminate the need for lifelong anticoagulant therapy. In addition to these properties they also provide resistance to the emergence of infections. The major disadvantage is the management of two valves during the replacement procedure for treating single valve disease. In some cases of failure of autograft, there is a chance for the patient to develop two valves diseases.

In biological tissue valves, time dependent structural changes leading to complication such as calcification, leaflet wear resulting in valve failure have been increasingly indicated by clinical experiences with aforementioned different valve designs. The main advantage of biological valves over mechanical valves is due to their low thrombotic risk and they do not require lifelong warfarin therapy (Bhuvaneshwar et al. 1991; Michael et al. 2007). Patients with bioprosthetic valves have a significantly decreased risk of bleeding when compared to the mechanical valves. But bioprosthetic valve also have some disadvantages causing a devastating impact resulting in significant morbidity and mortality. With the mechanical valve there is an increased risk of thromboembolism and infections like endocarditis but more durable. In contrast the bioprosthetic valve has decreased risk of thromboembolism and is less durable and frequently leads to valve failure (Dearani et al. 1997). The structural valve deterioration process of biological valves is not well elucidated. It is observed that the accumulation of both calcium and lipids on the surface of the valve is the reason for deterioration of the valve and also inadequate rinsing of the valve both prior and during the surgery process can also exacerbate structural valve deterioration which ultimately leads to an increased risk of calcification owing to the thickening of valve. The valve deterioration and associated calcification leads to malfunction of the valve thus affecting the pumping efficiency of the heart. The accumulation and deposition of calcium on the valve leaflets also result in narrowing of the valve called stenosis and leaflet tearing. Improvements on the bioprosthetic valves have reduced the onset of deterioration compared
with first generation valves. Despite all the improvements, structural valve deterioration remains a major drawback for the use of bioprosthetic valves. The structural deterioration of the valve leads to a high incidence risk of reoperation for patients implanted with these valves. One research study was conducted to compare the durability of bioprosthetic valves and mechanical valves, it was concluded that the possibility of reoperation is 25% for bioprosthetic valves and with 3% reoperation procedures for mechanical valves.

Bioprosthetic valves represent a far-reaching section for the surgical treatment of valvular diseases. These valves offer better hemodynamics without the need for anticoagulation therapy. However, durability of the valves remains a serious concern and several research works have been carried out over the past decades to address this issue (Banbury and Cosgrove 2001). Progress in the fabrication of biological valves include establishment of commendable models with new configuration, novel approach for treatment methods with various biological tissues, intensified preservation, and implantation techniques are effectively pursued. These efforts are made not only reduce the morbidity and mortality of the patients but also in reducing postoperative complications without compromising on the quality of life.

1.3.2 Mechanical valves

Mechanical heart valves are highly durable and provide longevity due to which it is preferred for patients under the age of 65 years for replacements (Jamieson 2002). Since the inception from 1950, there have been tremendous improvements made for mechanical heart valves over the past decades (Black and Durry 1994). The designs of mechanical heart valves include ball and cage designs. The first implanted heart valve had took place in the year 1952 (Starr and Edwards 1961). Tilting disc design was introduced in the year 1969 (Bjork 1971), bileaflet valve introduced in the year 1977.
(Emery et al. 1979) and are composed of two disc shaped leaflets made of Pyrolytic Carbon (PyC).

**Ball and cage valve design** consist of a metal cage. The ball used is made up of silicone elastomer occluder enclosed in a metal cage attached to a suture ring. Based on this model there are other caged ball type valves developed using disc as the occluder but are not used for replacements due to their poor haemodynamic characteristics.

In **tilting disc type valves**, PyC was used for the fabrication of disc that was fixed to a welded strut mechanism. The PyC and strut unit is attached to the suture ring. Due to the pressure difference created on either side of the valve, make the discs to tilt on its axis. As a result of tilting, there is a formation of minor and major orifice facilitating the flow of blood. As the valve closes, the reverse flow of blood is prevented and the unidirectional flow of blood is maintained. Based on the advantages of overcoming valve regurgitation problems tilting disc valves are preferred and number of improvements have been made with varying disc geometries and opening angles of the valve for enhanced unidirectional flow.

In **bileaflet valve designs**, there are two semicircular occluding leaflet is attached to the annular housing by hinges which is followed by attachment to the suture ring. When the blood flows through semicircular and rectangular openings, the leaflet opens for the blood to flow. The bileaflet model was the first to utilize all PyC for its design and it remains as one the most popular type of prosthetic mechanical valve for replacement, accounting approximately 80% of annually implanted valves for heart valve replacements (Michael et al. 2007; Edmunds et al. 1996).
The mechanical bileaflet valve designs namely St.Jude and Carbomedics were the most widely used valves for replacement procedures accounting for 85% of the total valves implanted (Peter 2002; Ozer et al. 2006). The advantage of bileaflet relies on their low profile which allows them to fit into smaller hearts during implantation without causing any obstruction to coronaries or mitral valves. Bileaflet valves offer better haemodynamic properties than the other mechanical valves used. The other advantages of bileaflet include high durability, low trans-valvular pressure gradient causing minimal valve incompetence and failure of the valve.

The Medtronic Hall and Monstrut Bjork – Shiley are the tilting disc valves and the second most implanted valves accounting only 7% of the valves implanted (Fabrizio et al. 2012). The advantage of tilting disc valves is due to their high durability. Unlike the bileaflet valves, tilting disc type valves are not ideal for better haemodynamics due to their effective orifice area and their turbulent flow around the disc. Starr-Edwards caged ball valve is the oldest valve and the least commonly used valves for replacement (Mehmet et al. 2005). These types of valves are not good enough in bringing better haemodynamic characteristic like the other two types of valves mentioned above. However, these valves are preferred under very difficult surgical
circumstances due to their advantage in easy handling and implantation. In spite of many developments made for mechanical heart valves, all types of valves developed faced a common problem i.e. partial obstruction in blood flow resulting in non physiological conditions for improved haemodynamic characteristics. A Non- physiological haemodynamic property of the implant material is the main factor that facilitates thrombus formation and related bleeding conditions, embolism and endocarditis infection. All these conditions often results in significant morbidity and mortality. The patients undergone with mechanical valve replacements have lifelong anticoagulation medications. These blood thinning medications pose to serious complications like bleeding and loss of circulating blood volume called haemorrhage which is frequently serious in some cases.

1.3.2.1 Evolution of mechanical heart valves

Dr. Charles Hufnagel, was the first to start the era of artificial heart valves in the year 1952 by successfully replacing total mechanical valve prosthesis (Lakshmi et al. 2009). This was performed by using a plexicas cage which contained a ball occluder, the cage with the ball was inserted into the descending thoracic aorta. After this pioneering effort, the first mitral valve replacement took place in the year 1960 by inserting the prosthesis in the mitral anatomical position (Copans et al. 1980). Starr-Edwards was the first caged ball type valve introduced for clinical use. After Starr-Edwards valve there are other similar designs based on caged ball appeared for clinical use like DeBakey–Surgitool, Smeloff–Cutter and Magovern–Cromie prostheses.

Caged ball designs also offer high durability; even though they are highly durable the central occluding design in the valve makes them to result in exerting high pressure and high turbulent stress across the valve. Many caged ball designs have been developed in the mid 1960s. Kay–Shiley, Beall caged-disc and Cross–Jones are introduced in the year 1965, 1966 and 1967.
respectively (Ibrahim et al. 1994). These three models developed were inserted in the atrioventricular location. But the implant fixed in these areas also resulted in high complication rates owing to the failure of valve and they soon fell into disuse. After the caged ball valve designs, there was a significant development in the heart valve prosthesis. The tilting disc valve by Bjork–Shiley was introduced in the year 1967. This model utilizes a free-floating disc located near the open position will tilt to an angle depending on the movement of the disc-retaining struts. It acts like an aerofoil in this position while the blood flows over and around it. This type of blood passage and movement will minimize the disturbance of the flow. The tilting disc based Bjork–Shiley prosthesis employed a polyactal occluder called Delrin for fabrication. Later delrin was replaced by PyC. Medtronic–Hall prosthesis incorporated with PyC was introduced in the year 1977 with successful outcomes (Antunes et al. 1988).

During the past few decades, different models have been developed incorporated with different materials were clinically evaluated on various stages. The ultra high molecular weight polyethylene occluder (namely Haynes-25 alloy cage) and sewing ring made of polyester was introduced for clinical application in the year 1990. The first bileaflet valve was introduced in the year 1978 by St. Jude Medical Inc (Emery et al. 1979). The bileaflet design consisted of two leaflets (semicircular-hinge) made of PyC. The occluder fixed in the open position will not obstruct the flow of blood and cause only minimum disturbance in blood flow. After the success of bileaflet valves, there are so many other valves developed based on bileaflet valve designs.

Development of Indian valve was initiated in the late seventies by the Sree Chitra Tirunal Institute for Medical Sciences and Technology, Trivandrum. The ChitraValve design was chosen to be of tilting disc type with a metallic cage, disc occluder and a sewing ring made of knitted
polyester fabric. During the decade of development, four models incorporating different materials were evaluated to various stages. The fourth model incorporating the Haynes-25 alloy cage, ultra high molecular weight polyethylene occluder and polyester sewing ring was introduced into clinical use in 1990 (Kalyani Nair et al. 2003).

1.3.3 Materials used for the fabrication of mechanical heart valves

The foremost design consideration of the object to be implanted in the system is the selection of materials. The materials must be competent to survive with the environment of the system. They have to be inert, biocompatible and should not bring forth any negative or refusal response. Factors to be considered for the heart valve prosthesis includes; material impact on hemodynamic property whether will it initiate platelet adhesion, aggregation and thrombus formation, damage of blood cells causing haemolysis, materials mechanical properties adequate to survive the repeated cycles of the flow. The materials required for the making of heart valves include PyC, titanium, graphite and polyester. For the outer ring titanium is the choice of material. PyC coated graphite is used for the leaflets. The inner ring of the prosthetic mechanical valve is made of 100% PyC. Silicon or tungsten is also impregnated with PyC for improving the durability. The double velour polyester is mainly used for the making of sewing cuff. The material that frequently applied for the suture ring is Dacron. Dacron is long chain polyester fabricated from terephthalic acid and ethylene glycol. Apart from Dacron, Teflon is another choice of material considered for the suture ring. Due to its low coefficient of friction it is applied in medical fields (Chambers et al. 2005; Toker et al. 2006; Teshima et al. 2003; Jamieson 2006).

The design and choice of material is the main engineering challenges for the fabrication of mechanical heart valves. The main evaluation
parameter taken into consideration for valve design is the choice of material, their haemodynamic performance, geometry of the valve like hinge design, shape of the leaflet and the opening angle. Materials considered for valve designing must suit numerous necessities include biocompatibility and chemical stability. Biocompatibility makes certain that the biological system can carry on implantation of the prosthesis without obliteration of tissue. Biological and chemical strength make sure that the material is defiant to degradation due to biological reactions with the body and environmental agents that encounter at some point during implantation. If the material used does not convince these requirements, infectious diseases such as endocarditis can builtin and potentially show the way for valve diseases leading to heart malfunction. Mechanical properties of the material must also be well thought of to make certain the durability and appropriate function of the valve.

Initial valve device intended to use metals such as stainless steel. Material fatigue is the cause to the led down of various replacement valves. Material fatigue is structural injure affected by cyclic loading of the material and possibly will lead to the creation of cracks and malfunction. The lower energy of materials used was investigated to replace with metal alloys and durable metals like titanium. However, the application of PyC as a material for artificial valves had overcome this problem to a certain extent. PyC is a biocompatible material with thrombo resistant properties which compose it appropriate for use in mechanical valve assembly and fabrication (Beugeling 1979). The material provides good stability, wear confrontation and potency, all of which are effective in reducing the mechanical failure of the valve (Fedel et al. 2010; Goodman et al. 1996). PyC was firstly used merely for the makeup of occluder discs, while the struts and housing are composed of metal alloys; with the initial bileaflet valves encompass an all-pyrolytic carbon design. Potential development to PyC is still an ongoing research; some modifications of surface engineering includes like covering
the material with nanocrystalline titanium oxide and diamond like carbon films (like DLC) to advance in its blood contact property (Fedel et al. 2010; Melanie et al. 2006). For this reason, the interface of surface geometry of the material with blood elements is also considered.

1.3.3.1 Application of Pyrolytic carbon as heart valve material

PyC is a synthetic material similar to graphite with covalent bonding between its graphene sheets and it exhibits unusual anisotropic properties. The graphene sheets crystallize in a planar order and thus have a single cleavage plane. It is more diamagnetic and thermally conductive along the cleavage plane. Pyrolytic carbon has homogenous, an isotropic and fine grained structure. It is a carbon material produced by pyrolysis of a hydrocarbon gas on suitable underlying substrates at a temperature ranging from 1000 to 2500K by a process called Chemical vapor deposition. The CVD process utilizes carbon sources such as hydrocarbons which include methane, carbon monoxide and acetylene. It is based on thermal decomposition at high temperature in a vacuum furnace which results in ultra pure product giving it a layered composition. It exhibits different properties in different planes. It has low thermal conductivity across its layers (C-Plane), acting as an insulator and very high thermal conductivity, and acting as an excellent conductor with the layers (A-B Plane).

It has a wide range of applications in nozzles and exhausts, solid fuel rockets and anti-oxidant coatings for silicon wafers. PyC is now widely used in medicine because of its unique properties like durability, wear resistance and biological compatibility with blood. Due to its excellent bio compatibility it is the choice for components in the majority of heart valves. This material can be coated on blood contacting prostheses to minimize the risk of thrombosis. In the heart valve industry, PyC is used to coat the external
blood contacting surface component parts such as a valve leaflets. DeBakey-Surgitool Aortic valve became the first valve to utilize PyC in its construction. A drawback to these early valves was their difficulty of maintaining central flow because blockage is not desirable for proper function.

1.4 CHALLENGES OF MECHANICAL HEART VALVES

The main merits of mechanical valves over other different types of artificial valves are their high durability. Due to different materials used and critical evaluation and testing of the material properties are highly concerned for the making of valves. Mechanical valves can last even lifetime of the patient. Due to these advantages mechanical valves are preferred over other varieties of valves in case of adolescent and paediatric patients (Pibarot et al. 2009; Michael et al. 2007). However, they are also associated with problems owing to serious complications. The other issues considered for mechanical valves involve both biological and engineering module. When artificial material implanted in the body composed of living cells, the important factor to be measured is the interaction of the system and the mechanics of the valve. When artificial heart valves are implanted, the responses initiate cascades of biological events like the formation of blood clots. These adverse reactions may also result in valve malfunction leading to valve failure. Several other factors that contribute to major complications as a result of implantation of prosthetic heart valves are discussed below.

1.4.1 Cavitation

Formation of vaporous bubbles in a fluid rapidly is called cavitation. This is caused by a local pressure difference below the fluid vapor pressure. When the pressure difference occur the nuclei or the microbubbles will enlarge and creates the formation of bubbles. The formed bubbles will collapse as the pressure recovers causing pressure and thermal shock waves
that damage the solid material across the site of cavitations. This type of
damage can cause cavities on the leaflets or housing of the prosthetic valve
material implanted. This process is referred to as pitting or erosion. Flow
deceleration, pressure oscillations, tip vortices along with squeeze jets are the
main causes for creating pitting or erosion on the valve material. Sometimes
the fluid between the valve housing and occluder gap fluid is pushed off
resulting in the formation of high speed jet. This is then mixed with the fluid
outside the gap and also over the two layers of fluid further resulting in
pressure difference. This high speed jet formation results in the formation of
tip vortices. The speed jet and the tip vortices created are the major factors
contribute to the process of cavitation.

1.4.2 Prosthetic valve thrombosis and embolism

The incidence of prosthetic valve thrombosis (PVT) has been
reported by Pibarot and his coworkers in the year 2000, with an average of
0.2% and 1.8% per patient-year after aortic and mitral valve replacement
respectively. Raymond Roudaut and his coworkers in the year 2007 reported
high prevalence of fibrous and pannus formation with associated risk of
thrombosis in 45-75% cases of heart valve replacement procedures. Horstkotte and his coworkers in the year 1995 reported that the incidence of
obstructive PVT and thrombosis and embolic complications for mechanical
valves varies between 0.3–1.3% and 0.7–6% patient years. Deviri and his
coworkers in the year 1991 also reported a 24% incidence of thrombosis in
the first post operative period of surgical interventions of PVT.
Figure 1.5 Schematic representation of thrombosis and embolism (http://www.heartvalveresearchuk.org/)

Thrombosis is major issue causing adverse effect with the mechanical valves. Thrombosis refers to the initiation of forming a stationary thrombus and the formation of blood clot along the blood vessel wall. Sometimes the strong blood clot may detach from the wall of blood vessel and leads to a condition called embolism. Embolism is also another serious issue that leads to the obstruction of a blood vessel in the system. They may also contribute to complications like myocardial infarction or blockage of artery in the brain called ischaemic stroke. The risk of anticoagulation-related complications is between 0.3 and 1% a year with a reported mortality of 50% within first 30 days (Raymond et al. 2007). Prosthesis related problems occur within 10 years of valve surgery in 30–35% of cases in patients under mechanical valve replacements (Oxenham et al. 2003). Blood clot formation, detachment of clots from the blood vessel is a serious issue after valve replacement results in endocardial damage, chamber enlargement, weakened cardiac function and associated irregular heart beat condition called arrhythmias. The thrombus formation is a highly complex process because it
involves many factors and processes. Damage of blood elements causing haemolysis and rupture of platelets will initiates two interconnected processes namely the coagulation cascade and platelet activation. These two processes results in the formation of a strong blood called thrombus. Damage of the endothelial cells is due to the extended shear stresses thereby collagen is exposed near the vessel wall that was damaged. Due to injury and blood contact with the exposed collagen will result in the release of tissue factors and the activation of coagulation cascade. During this process of establishment, adenosine diphosphatel (ADP) are allowed to get released which in turn will activate platelets; this activation will further activate other platelets contributing to synergistic effect. The activated platelets and other tissue factors attach to each other near the injury site forming a loose plug for the prevention of blood loss. Coagulation process also involves a complex set of chemical reactions for the production of enzyme called thrombin. This will activate platelets, which further converts the soluble protein in blood (fibrinogen) to an insoluble (fibrin) protein. The mesh of fibrin network formed across the injury site will further trap the activated platelets and red blood cells for the stabilization of loose plug.

Blood flow in prosthetic mechanical valves downstream locally is not entirely physiological condition. This is also due to the valve design and materials used for fabricating them. Because of which the prostheses are inducing extremely high shear stresses of blood, further resulting in stagnation and flow separation leading to the formation of thrombus. The closed position of the valve will not guarantee a proper seal may induce some leakage causing regurgitation. This mainly depends on the materials properties for the fabrication of mechanical valves. The leakage in blood flow at the hinge regions is observed on the bileaflet valve. Same type of effect is observed between the housing and leaflet edges through the gaps in tilting type valves. When this type of leakage occurs there is high stress exerted on
the elements of blood leading to cells damage called haemolysis and thrombogenesis due to rupture of platelets.

1.4.3 Haemorrhage

Patients are given with lifelong anticoagulant medications due to associated risk of thrombosis and embolic with mechanical valves. The oral anticoagulation (OAC) therapy will minimize the formation of thrombus. There is also an increased risk of haemorrhage associated with OAC medications. Although many improvements are implemented in the mechanical valve designs to reduce the amount of blood thinning medications, anticoagulation induced haemorrhage is still a major issue for heart valve replacement (Blackstone et al. 1995, Butany et al. 2003).

1.4.4 Pannus

Pannus is the formation of excess tissue growth across the suture ring area which is used to attach the valve in place. The pannus overgrowth condition can obviously result in narrowing of the valve orifice owing to serious complications. This condition may lead to the immobilization of leaflets. Pannus growth is considered to be one of the main conditions of obstructive valve failure.

1.4.5 Endocarditis

Prosthetic valve endocarditis (PVE) is the infection of the heart valves or the heart’s inner lining caused by a variety of bacteria and fungi, associated with serious effects with a reported occurrence from 1% to 9.4% of patients (Masters et al. 2001). It is an endovascular, microbial infection, most common with prosthetic valves with a frequency ranging from 0.1–2.3% per patient year (Gnann et al. 1983). Infective endocarditis is an unchanging incidence approaching a mortality of about 30% at 1 year (Groves et al.
2001). Prosthetic heart valve related problems affects normal blood flow through the heart are at high risk for endocarditis. Patients at the risk of developing endocarditis are advised to be on antibiotics as a preventive measure for the disease. *Staphylococcus aureus* and other gram positive bacteria like *Streptococcus viridians* contributes for nearly 30% of IE associated with prosthetic valves. Gnann and his coworkers in the year 1983 reported the incidence of infection of about 2.5% on intracardiac prosthesis after valve replacement. Early PVE occurs with an onset within 60 days for approximately one-third of all cases and the remaining two-thirds accounts for late PVE which occurs more than two months after valve replacement. In patients with valve replacement, there is 1% to 3% incidence of endocarditis after valvular heart surgery (Blackstone et al. 1985).

![Figure 1.6 Mechanical heart valve complications (A) Thrombosis on valve (B) Endocarditis on valve (C) Pannus formation on valve](http://www.heartvalveresearchuk.org/)

1.5 THROMBUS FORMATION ON MECHANICAL HEART VALVES

The coagulation cascade consists of two separate initial pathways namely the intrinsic and extrinsic that eventually congregates on the common pathway. These two pathways fundamentally serve to activate the protein prothrombin to thrombin. The intrinsic pathway comprises the contact
activation system. The extrinsic system is the primary initiating pathway of blood coagulation in physiological environment; engaging both blood and vascular elements. The significant component is called thromboplastin, a glycoprotein entrenched with phospholipid in the surface membrane of fibroblasts. Under in vivo surroundings, the tissue factor are not exposed to blood, however by means of injury or damage to vascular or endothelial tissue will accomplish the process. This factor will get activated along with other tissue factors. The intrinsic pathway can be a scrutiny, as coagulation is instigated by components that are completely contained within the vascular system. This pathway results in the commencement of tissue factors that endow with blood coagulation.

Figure 1.7 Schematic representation of platelet aggregation forming a strong blood clot(http://www.heartvalveresearchuk.org/)

Integrated in the intrinsic pathway is the contact system where skin, muscle and connective tissue and a range of additional surface will possibly acts as activators. The responsibility of contact system proteins in the instigation of the intrinsic pathway of haemostatis process is uncertain. The proteins involved in hemostasis process is dependent on number of additional events like complement activation, inflammatory reaction and fibrinolysis and
are moreover serious when blood act together with a foreign surface. The fibrins molecules will build up uniformly by trapping platelets, erythrocytes, and leukocytes for the thrombus formation. The clot subsequently contracts in a collective manner near the boundaries of the wounded surface. A clot that remnants in the region where it developed is called thrombus and the responses circumciet for the formation of thrombus is called thrombosis. In region where a thrombus has fashioned there is also a tendency for the clot to augment in size. As a result the blood flow will sluggish around the thrombus created area. Clot forming essentials like platelets, red blood cells, and clotting factors are set down by generating an extend or propagating thrombus.

1.6 COAGULATION PROCESS

The progression by which the body averts loss of blood is called coagulation. Coagulation engages the development of a blood clot referred as thrombus so as to stop additional loss of blood from injured tissues or any blood vessels or from any organs. The blood coagulation is a complex procedure with a cellular system encompasses of cells called platelets that flow in the blood and function to form a platelet plug above injured vessels. The system is based upon the accomplishment of numerous proteins called as clotting factors to generate a fibrin clot. Any disorder in the system will cause excessive clotting or diminutive clotting. Platelets are essential for serving three primary functions i.e. fixing to the wounded blood vessel called as platelet adherence, sticking to additional platelets to expand or broaden the plug formation referred as platelet aggregation, ensuring support as molecules on the exterior of platelets are obligatory for various responses for the coagulation process. When blood vessel rupture takes place, many factors exposed to the elements that are not in direct contact with the blood flow. These substances mainly collagen and von Willebrand factors permit the platelets to stick to the affected surface. When platelets are attached to the surface they will liberate chemicals that draw other platelets to the injured area resulting in aggregation. These two schemes are the primary reaction to
arrest bleeding. The coagulation process is a protein based systems that become stable to serve clot that has been created and promotes to seal up the wound.

Figure 1.8 Schematic representation of classic intrinsic and extrinsic coagulation pathways

The aim of the coagulation cascade is to form fibrin which resolves to form a network contained in the platelet aggregates to maintain the clot. All the coagulation factors will have active and inactive forms. In activated form they will serve to trigger the next factor in the series till fibrin is formed. This process will take place where injury or damage occurred to form platelet aggregate. Tissue factor and factor VIIa where ‘a’ is the active form of the factor; will trigger factor X and forms factor Xa. Factor Xa will then stimulate prothrombin (factor II) to produce thrombin (factor IIa).
Thrombin will then change fibrinogen (factor I) to fibrin (factor Ia) respectively. Fibrin forms a mesh along with the activated platelets forming a plug and fibrin mesh is further stabilized by factor XIII forming the strong clot (formation of an intricate network of cross-stitched strands of fibrin). The factors that hinder or accelerate the coagulation process includes Factors V and VIII, which will trigger the conversion of factors namely X to Xa by the action of factor IXa (this is carried out by factor VIII) and further conversion of prothrombin to factor IIa by factor Xa. The factors that restrain the clot formation steps include; Protein C and S, thrombo-modulin complex activated by factor IIa. The role of these factors is the expansion of complicated group of proteins with the function of inactivating factor V and VIII. Antithrombin initially called as antithrombin III contend to obstruct the actions of several clotting factors.

1.6 INTRODUCTION TO NANOTECHNOLOGY

Nanotechnology is up-coming as a fast emergent field with its function in science and technology for the reason of developing innovative resources at the nanoscale level. It concerns to the invention, design and function of well-designed structures with one characteristic dimension less than 100 nanometer (Grainger and Castner 2008). The term ‘nano’ is applied to denote one billionth of a meter or $10^{-9}$. The word Nanotechnology was invented by Norio Taniguchi, Professor of Tokyo Science University during the year 1974 to portray exactitude development of materials at the nanometer level (Huh et al. 2007). The onset of Nanotechnology was specified by physicist Professor Richard P. Feynman in his talk ‘there’s plenty of room at the Bottom’ (Laval et al. 1999). Nanoparticles are group of atoms in the size array of 1–100 nm. ‘Nano’ is a Greek expression to dwarf meaning very little or small. The use of nanoparticles is ahead force existing in the current century as they acquire definite chemical, optical, mechanical and biological characteristics. This material arrangement exhibits unique and widely
improved physical, chemical and even biological properties, because of their distinct structural features which involve between isolated atom and the bulk microstructures. This is a novel field which is all set to transfigure the fundamental skeleton of surface engineering and coating science (Li et al. 2009). Nanotechnology has tremendously been the wide choice of potential application from nanoscale electronics and optics to nanobiological systems, nanomedicine and novel materials. Diverse feature of this technology include (i) creation and processing of materials and structures in the nanoscale, (ii) understanding the physical properties related to the nanometer scale, (iii) invention of nanodevices or devices with nanomaterials as building blocks and (iv) design and creation of original tools for classification of nanostructures and nanomaterials. Incorporation of nanoparticles as surface coatings on biomedical devices will enhance its biocompatible properties especially heart valve prosthesis to overcome thrombosis and infections (Moghimi et al. 2005; Tang et al. 2007; Melanie et al. 2006; Liu et al. 2007). Surface coating technologies can also have a profound impact on manufacturing processes and provide potential opportunities to enhance performance and improvements in a number of biomedical applications and its functional properties. The metallic nanoparticles are most promising candidates in biological applications as they demonstrate superior antibacterial properties owing to their huge surface area to volume ratio.

Different variety of nanomaterials like copper, zinc, titanium (Ahmad et al. 2003), alginate (Albrecht et al. 2006), magnesium, gold (Ahmad et al. 2003) and silver have come up for their potential role in many different applications. But silver nanoparticles have been well established and also found to be most effective as it has superior antimicrobial efficiency in opposition to bacteria, viruses and eukaryotic microorganisms (Pradeep et al. 2005). Silver nanoparticles as drug antiseptic encompass several hazards as the contact to silver can leads to a condition called agyrosis as well as argyria.
Silver is toxic to mammalian cells (Gong et al. 2007). Use of metallic silver or silver ion as well as AgNPs can be functional in medicine for burn treatment, dental materials, water treatment, coating stainless steel materials, textile fabrics, etc. They also possess low toxicity to human cells, high thermal stability and low volatility (Kim et al. 2008).

1.8 POTENTIAL ROLE OF SILVER IN BIOLOGICAL APPLICATIONS

The properties concerned with antimicrobial activity of silver (Ag) have been acknowledged for thousands of years. In prehistoric times it was used in water containers and to avoid rotting of fluids and foodstuff. In olden times, water and milk were reserved in silver coated vessels. Silver was furthermore mentioned in the Roman pharmacopoeia of 69 B. C. At the commencing of last century, Gibbard (Kumar et al. 2008) was the principal inventor to methodically explore the antimicrobial properties of silver.

Silver nitrate is the most widespread silver compound that finds its application as an efficient medicine in clinical practice. There is strong confirmation in the text that the silver is the active constituent of silver salt. Silver nitrate is a substance that facilitates the release of silver ions quickly. The antimicrobial action of silver ions dissolved as silver nitrate was premeditated in diverse studies. The bactericidal capability of silver at a range of concentrations (0-0.1 mg/L of Ag+) against a variety of bacterial strains namely Legionella species and gram negative Pseudomonas aeruginosa and Escherichia coli was tested. The results showed that silver demonstrated satisfactory bactericidal capability to inactivate bacteria’s tested at different concentrations without influencing the quality of drinking water or mammalian cells (Huang et al. 2007). The parameters with special consideration to CIC, PAE, CAE, and MBC, as an indication for the quantitative investigation of the receptiveness of bacteria to silver ion was
well elucidated (Chopra 2007). The utilization of silver nitrate for the management of pathogens causing periodontal disease and associated complications is studied in detail (Denning 1991). They established that silver nitrate was more competent than antibiotics for the cure of periodontal contamination with infectious organisms. In addition, number of researchers investigated the use of Ag+ electrically. Berger and his coworkers in the year 2000 determined the bacteriostatic and bactericidal concentrations (MIC, MBC) of electrically produced silver against 16 experimental test pathogens and they confirmed that Ag+ particles synthesized were found to be extremely efficient without causing adverse effects (Kim et al. 2008).

1.8.1 Various mechanism of antimicrobial action of silver

The accurate mechanism of action of silver on the microbes is still not known but the possible mechanism of action of metallic silver, silver ions and AgNPs have been elucidated based on the morphological and structural changes found in the bacterial cells. The most widely known bactericidal mechanism of the silver ion is due to their interaction with the L-cysteine (thiol group) residue of proteins and consequent inactivation of their enzymatic functions. Feng and his coworkers in the year 2008 studied the effect of silver nitrate against two strains of bacteria (S. aureus and E. coli) in LB medium by transmission electron microscopy (TEM) and X-ray microanalysis (Kim et al. 2008) and demonstrated a possible mechanism of action of silver ions. The actual mode of silver ions entry to the bacterial cell is through the penetration of cell wall and consequently turns the DNA into condensed form. The condensed form of DNA will then which reacts with the thiol group proteins, resulting in cell death. The silver ions also interfere with the replication process. Using an electrochemical method, the antimicrobial effect of micromolar concentration of AgNO3 (≤10µM) against E. coli was reported. They observed that the rate of respiration of E.coli increased initially. Addition of silver ions caused uncoupling of the respiratory chain,
followed by cessation of respiration. Even low concentrations of Ag+ induced a massive proton leakage through the cell membrane, which resulted in absolute deenergization and cell death (Kumar et al. 2008). The antibacterial efficacy of silver ions was investigated using \textit{E. coli} as a representation organism with the help of techniques namely energy-filtering TEM (EFTEM) and 2D- electrophoresis, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) facility. From the study it was concluded that bactericidal action of silver ions was basically due to the silver ion interaction with ribosome and the suppression of enzymes and proteins necessary for ATP production. Paland his coworkers in the year 2007 studied the bactericidal activity against \textit{E.coli} and \textit{S. aureus} (Kalimuthu et al. 2008). They demonstrated that antimicrobial activity of silver ions was closely related to the presence of oxygen that results in the generation of reactive oxygen species (ROS).

The effect of silver ions on bacteria can be observed by the changes in structure as well as morphology. It has been reported that, when DNA molecules are in relaxed state the replication can be effectively conducted. But the DNA in its condensed form will lose its replication ability. When the silver ions enter inside bacterial cell through penetration the DNA molecule turns into condensed form and loses its replication property affecting the cell growth. Also, it has been reported that silver react with proteins by getting attached with the thiol groups resulting in inactivation of proteins owing to the loss of viability (Dibrov et al. 2002; Kim et al. 2008).

The silver nanoparticles demonstrated proficient antimicrobial property when compared to other groups due to their very large surface area which offer improved interaction with microorganisms. The nanoparticles have the ability attaching to the cell membrane as well as enter into bacteria. The bacterial membranes have sulfur rich proteins. The AgNPs intermingle with these proteins in the cell and also with the phosphorus rich compounds
like DNA. Whilst silver nanoparticles go into the bacterial cell after penetrating the membrane, there is a formation of low molecular weight area in the core of the bacteria to which the bacteria are firm thereby protecting the DNA from the silver ions. The nanoparticles possibly attack the respiratory chain of bacteria, their cell multiplication and finally cause cell death. The nanoparticles will discharge the silver ions into the cells of bacteria which is responsible for their efficient bactericidal activity (Feng et al. 2000; Sondi and Salopek-Sondi, 2007; Duran et al. 2007; Song et al. 2006).

Figure 1.9 Schematic representation of mechanism of antibacterial action of Silver nanoparticles (AgNPs) (Gianluigi et al. 2015)

Sondi and his coworkers in the year 2004 described the antimicrobial efficacy of silver nanoparticles on *E. coli* as a representation for gram negative bacteria. From the SEM study, they observed the pattern of silver nanoparticles aggregates formed and the significant bacterial cell death. They also found that the silver nanoparticles interrelate with the construction elements of the membrane of bacteria and result in injure to the cell. The TEM and EDAX analysis established the integration of silver nanoparticles
into the membrane which was shown by creation of pits on the surface of the cell membrane. They demonstrated that silver nanoparticles proceed mainly in three ways against gram-negative bacteria; (1) nanoparticles get attached to the cell membrane surface and perturb its appropriate function like respiratory function and cell permeability; (2) they are proficient to enter inside the bacterial cell membrane and lay foundation for damaging the compounds that contains sulfur and phosphorous by intercalating with them; (3) nanoparticles will liberate silver ions which will further contribute to the efficient antibacterial action of silver nanoparticles.

Lok and his coworkers in the year 2006 produced spherical shaped AgNPs with an average particle size of ~9.3 nm particles using a borohydride chemical reduction technique. They explored their antibacterial activity against *E. coli* by 2D electrophoresis proteomic approaches. The proteomic information demonstrated that a limited exposure of *E. coli* to AgNPs resulted in the accumulation of envelope protein precursor further elucidated the dissolution of proton motive force. Unfailing with these proteomic conclusions, AgNPs was revealed to weaken the outer membrane, disintegrate the plasma membrane potential and minimize the amount of intracellular ATP.

In addition, the antimicrobial activity of AgNPs was dependent on their size and shape of the synthesized particles. Pal and his coworkers in the year 2007 examined the antimicrobial effect of various shapes of nanoparticles and its activity against gram negative *E. coli* both in nutrient broth and in nutrient agar plate. Curtailed triangular shaped silver nanoplates demonstrated very strong antibacterial action in contrast with sphere shaped and rod shaped nanoparticles. Upon bacterial treatment with AgNPs, energy filtering- TEM images showed significant alterations in their cell membranes and consequential cell death. Elechiguerra et al established that AgNPs undergo a size contingent contact with human immunodeficiency virus (HIV)
type 1 probably by means of binding to gp120 glycoprotein protrusion. The same group confirmed that nanoparticles to facilitate undeviating contact with bacteria preferably encompass a diameter of ~1-10 nm.

In literature many works have explored the addition of silver as antimicrobial agents into a variety of matrices. AgNPs implanted into a matrix of amorphous silicon dioxide permeated in bacterial cellulose showed better antimicrobial activity (Mukherjee et al. 2001). Some significant merits of silver based antimicrobials are their exceptional thermal effectiveness and their strength and ecological safety. The mechanism concealed with the antibacterial effect of silver impregnated materials is still unsure. Some information have recommended that the antibacterial efficacy of silver incorporated materials is recognized by means of elution or leaching of silver ions into the system encompassing microorganisms leading to cell death. Cell death is accomplished by penetrating into cell membrane and binding at definite sites to DNA, RNA and targeting respiratory enzymes and other cellular proteins (Pal et al. 2007). Several other factors also attribute to the antibacterial activity of silver incorporated materials which include catalytic oxidation concerning the formation of Reactive Oxygen Species (ROS).

Patients who are mechanically ventilated are inserted with endotracheal tubes are at greater risk of acquiring pulmonary infection caused by bacteria inherent in the hospital. One method to reduce the hazard is to make the surfaces of endotracheal tubes antibacterial with a mean to avoid bacterial adhesion to a maximum extent to prevent infection. Russel and his coworkers in the year 1994 impregnated silver onto the endotracheal tube surface by a technique called wet chemical treatment. The authors found that the bacterial growth on treated surface was tested by gram negative *Pseudomonas aeruginosa*. Bacterial intensification tests revealed that the surface modified by wet chemical treatment method obstructed bacterial growth not only on the
particle area of exposure but also in the background of the tube which may be possibly due to leaching of silver ions.

Devices used for implantation procedures especially mechanical heart valve prosthesis are at a foremost risk for hospital-acquired infection. Biomaterials that are coated with either silver oxide or silver alloys have been in practice for reducing infection. Furno and his coworkers in the year 2004 established a novel approach by means of adopting silicone for the impregnation of metallic silver nanoparticulates. They accomplished, that this impregnation method presented two merits; one is continuous release of silver ions at a concentration possessing greater antimicrobial activity and the other is due to their capability to guard both inner and outer surfaces of catheters.

Pyrolytic carbon has been extensively used in cardiovascular surgical procedure as artificial heart valves due to its noteworthy biocompatibility and mechanical properties. Tang and his coworkers in the year 2008 examined the biocompatibility and antibacterial efficacy of Ag+ implanted PyC. The PyC samples were impregnated with silver ions at an energy of 70keV with varying the dose from 5*10^{14} to 5*10^{18} ions/cm^2. The results confirmed the antibacterial effect of Ag implanted PyC for both gram positive *S. aureus* and gram negative *E. coli*. The rate of antibacterial activity is increased with an increase in ion dose. The silver ion dose prepared at 5*10^{17} ions/cm^2 showed the most promising antibacterial effect with a bactericidal rate of 97%. It was obvious from the results that the Ag+ implanted PyC possessed a very strong biocompatibility devoid of toxicity.

Silver nanoparticles are also recognized as a suitable material for coating medical devices (Roe at al. 2008). Medical devices covered or layered with silver ions or metallic silver provided evidence to be satisfactory in some
clinical tests. The cause for this may be due to the activation of metallic silver when it gets in touch with blood components and also due to the efficiency to maintain coatings stability. Furno and his coworkers in the year 2004 confirmed the application of AgNPs into polymeric medical devices augmented their antimicrobial efficacy to several folds. Thus AgNPs can be used for coating of medical devices and lead to show potential antimicrobial activity (Li et al. 2006). The benefit of AgNPs coating of medical devices will defend both outer surfaces as well as inner surfaces of devices and there is a constant and uninterrupted release of silver ions owing to antimicrobial activity (Wilcox et al. 1998; Darouiche et al. 1999).

1.9 POTENTIAL ROLE OF COPPER IN BIOLOGICAL APPLICATIONS

The accessibility of copper made it a superior option to exert with, since it shares some characteristics comparable to those of various exclusive noble metals like silver and gold. The wide utility of copper by humans date backs to 5th and 6th B.C. It appears that this was the foremost metal to put in use because of its native metallic form and which did not have the need of smelting unlike other noble metals. The oldest proof of medical application of copper is pointed out in the Smith Papyrus, the egyptian medical manuscript wrote between 2600 and 2200 B.C (Raffi et al. 2010). It is mentioned that copper is employed to disinfect or fumigate chest wounds and also for drinking water sterility. Romans, Greeks, Aztecs and various others also utilized copper and copper based compounds for the management of complaints such as headaches, intestinal worms, and burns, treating ear infections and also applied for basic sanitation. In the 19th century, understanding of copper's medical effectiveness and strength was initiated by the surveillance that copper workers protected from cholera in the year 1832.
Utilization of copper in medicine turned out to be extensive only in the late 19th and early 20th centuries. A multiplicity of research was carried out based on inorganic copper for the management of chronic adenitis, tubercular infections, impetigo, eczema, lupus, facial neuralgia, scrofulosis, syphilis and anemic conditions. The application of copper as an antimicrobial component sustained until the introduction of commercially existing antibiotics in 1932 (Lee et al. 2013). The increase of antibiotic resistance in recent times has made antibiotic defiant bacteria ever present in hospitals, animal breeding amenities and food processing plants. This has lifted up the requirement for a choice of advances to carry out emergent research for the prevention of microorganism invasion and subsequent infection. One such option is the application of copper on exterior surfaces in specific susceptible areas (Chatterjee et al. 2012). While this advance is not novel, it had nowhere to be found significance and recognition in the most recent decades.

Copper Nanoparticles (CuNPs) have been noted for their antimicrobial efficacy against variety of bacterial and fungal species. Materials measured within the range of 100 nm or below are considered to be in nanoparticulate forms. They display a broad range of characteristics such as optical, catalytic, magnetic, electrical and biological properties that differ from bulk counterparts. Several biotic distinctiveness of nanoparticles have been investigated by testing the antimicrobial tendency of nanoparticles fashioned from diverse metals by means of special synthetic techniques. It has been showed that various metal nanoparticles such as Au, Ag, Cu and CuO demonstrated a broad range of antimicrobial exertion against various classes of microbes i.e. fungi, gram-positive and gram negative bacterial species (Lee et al. 2013). Unwarranted release of silver for instance may cause environmental contamination which in turn formulates silver destructive to
humans and animals (Gaggelli et al. 1995). Copper is no exemption, since surplus of copper in the human body show the way to production of the most harmful and destructive radicals such as the hydroxyl radical. On the other hand, few copper transporting enzymes such as adenosine triphosphatases (Cu-ATPases) as well as ATP7A and ATP7B participate as a significant function in copper homeostasis and release excess amount of copper through the intestine (ATP7A) as faeces, the ATP7B in liver as a bile product, and the (ATP7B) mammary gland as milk (Lutsenko et al. 2007). Still CuNPs comprise major restrictions which include their quick oxidation on contact to air. Copper oxidizes to CuO and Cu₂O and furthermore translate to Cu²⁺ at some stage in preparation and storage. Consequently it is hard to produce CuNPs in an ambient atmosphere.

1.9.1 Various mechanism of antimicrobial action of copper

Copper coated surface have an effect on bacteria in two chronological steps; the initial action is through contact between the outer membrane of bacteria and the treated surfaces causing the bacterial covering i.e. the membrane to rupture and release of cellular contents. The next is connected to the puncture or creating holes in the external membrane in the course of which, the cell loses essential nutrients along with water. This will lead to a wide range of deterioration of the cell. The external membrane of a single cell organism like bacterium is categorized by a constant electrical micro-current. This property is referred to as "trans-membrane potential" and factually a voltage disparity occurs between the outside and inside of a cell (Lee et al. 2013).
It is believed that as soon as a bacterium moves towards in contact with a copper surface, a small circuiting of the current in the cell membrane will take place. This deteriorates the membrane and generates the formation of holes. An additional way to create a hole in a membrane is through process of local oxidation or corrosion. This takes place as soon as a copper particle or a copper ion is leached or released from the copper surface and strikes a construction block of the cell membrane whether a protein or a fatty acid. The strike or hit process will occur in the presence of oxygen and results in the oxidative injure or corrosion. The major resistance of the cell is provided by their intact cell membrane. The external covering of the bacteria is ruptured and copper ions will enter into the cell. This will obstruct the function of various cellular components within the cell. Copper literally engulfs the contents inside the cell and subsequently obstruct cell metabolism such as targeting biochemical enzymes needed for various processes. These reactions
are accomplished and catalyzed by various enzymes. When there is an excess amount of copper they get attached to these enzymes that are involved in various cellular processes. With further treatment the bacterium will not be able to respire, consume, assimilate or generate energy (Ruparelia et al. 2008; Wei et al. 2010).

1.10 INTRODUCTION TO DEPOSITION TECHNIQUES

Deposition of thin films is a difficult problem for quite a number of reasons. The deposition method have to be satisfactorily established to deposit nm range thin coating over a extended sufficient time that is usually stable. Based on the uniqueness of materials and the cost issues, numerous techniques have been engaged for the development of growth of thin film and multifaceted coatings. The methods adapted for thin film deposition include Thermal Deposition (TD), Sputter Deposition (SD) and its modification like DC, RF, Magnetron, medium frequency magnetron technique; Chemical Vapor Deposition (CVD) technique and its modified methods (Plasma Enhanced CVD and Low Pressure CVD). Ion Beam Deposition, Pulsed Laser Deposition (PLD) and their combinations are well studied methods for the fabrication of thin film coatings. Thermal deposition method is one of the oldest and most available techniques to produce thin films. Thermal deposition and Molecular Beam Epitaxy (MBE) are predominantly valuable for growing superior quality metallic and semiconductor films in super-lattice pattern. The CVD technique adopted to develop films on a substrate by decomposing a selected precursor vapor and is highly favored in engineering applications for its superior and spatially uniform growth rates. However, the elevated deposition high temperatures engaged in CVD technology disqualify the use of this technique in several applications. The other technique that has been applied very often is reactive magnetron sputtering. Reactive sputtering is favored over sputtering commencing a compound target to attain advanced deposition rates. But this pretense the challenging problem of depositing two
materials concurrently necessitates diverse partial pressures of a reactive gas. The minimal energies of the depositing material direct to the occurrence of porosity at the grain boundaries owing to inflexibility. In contrast to these growth techniques, PLD is advantageous and versatile technique that finds application as a means of patterning extremely varied choice of materials and in extensive areas of thin film coatings and multi layer exploration. The methodology of PLD engaged by centering a powerful laser pulse on the exterior of a target and removes the material used as targets in the form of volatile stages i.e. gas or plasma. The distinctive merits of PLD is due to its ease and simplicity in view of the fact that the laser is entirely decoupled from the growth compartment or chamber, their capability to conserve the materials stoichiometry under most favorable conditions.

1.10.1 PULSED LASER DEPOSITION

PLD is a unique technology for both thin and thick films material growth. This technique is able to yield rapid fabrication of device superior functional materials. In the most recent years, PLD has developed as one of the most accepted and inherently straightforward techniques for depositing a broad range of unique and novel materials (Smausz et al. 2006). This is currently being investigated for third generation coatings for widespread applications. Such continued recognition is owed to the intrinsic adaptability, flexibility and rapidity of a process that can be applied fundamentally to deposit several materials like metals, alloys, polymers, insulators and possibility of coating even biomaterials) upon any substrate (Hopp et al. 2007; Kecskeméti et al. 2006). Progressed in the late 1986 with the invention of high Tc superconducting material (HTS), the method of PLD was rediscovered from its original manifestation during mid 1960’s with subsequent development of high power lasers. At present this innovative methodology is being implemented to the extended range of materials growth
starting from single atomic coating to quasi-bulk crystalline materials by means of thickness beyond 100 nm. This technique is also effectively used to formulate numerous varieties of multi-component thin films attained with superior quality. However, the technique also associated with some restrictions; like the formation of particulates, discordant ablation. Deposition of materials over huge areas can be minimized by integrating some alterations to the conventional PLD method.

1.10.1.1 Description of the coating processes

The technique of PLD comes under the physical vapor deposition process executed in a vacuum system. This technique shares a few common technical characteristics with sputter deposition and molecular beam epitaxy. In this method, a pulsed laser is focused to the material to be deposited known as target material. With adequately high laser power density, each one of laser pulses will vaporizes or ablates a little amount of the target material and creates plasma which is known as plume. At the initial phase of the laser pulse interaction, an intense layer of vapor is created in front of the ablating material i.e. target. Energy absorption during the memento of the laser pulse makes both the pressure and temperature of the vapor to enhance and creates plasma plume. The ablation plume developed consists of ablated material which is extremely frontward directed irrespective of the position of incidence of the laser beam and offers the material flux for thin film growth and deposition.
1.10.1.2 Salient features and important process parameters

The salient feature of PLD process is their capacity to comprehend stoichiometric transfer of the ablated material from multi-cation object for an extensive choice of materials. This occurs from the non-equilibrium state of the ablation development owing to the absorption of high laser energy density through a little amount of material. For low absorption the laser will just heat the target with ejected flux due to target species thermal evaporation. In this situation, the evaporated flux from the target might be dictated by the vapor pressure of the components. As the fluences is enlarged further than an ablation threshold, the energy assimilation found to a great extent than the desired thermal evaporation. The ablated species take up the remaining laser power resulting in vaporization that does not reliant on vapor pressures of constituent cations. This causes similar ablation that make certain for
stoichiometric transfer. The next significant feature of PLD process is their rate of deposition. In laser ablation, every ablation pulse determines to offer material adequate for the deposition of sub monolayer of the preferred phase. The quantity of film expansion for each laser pulse is based on several factors including target-substrate distance, surrounding chamber environment gas pressure, laser spot dimension and laser energy density. Under characteristic circumstances, the rate of deposition for each pulse can vary from 0.001 to 1 Å and for this reason PLD facilitate laser shot-to-shot management of the deposition process that is superlative for multilayer and interface patterning of thin films.

1.11 SCOPE OF THE THESIS

Pyrolytic carbon is a synthetic material and possesses good strength, durability, biocompatible and thrombus resistant properties which make it ideal for their use in mechanical valves. The initial use of PyC was limited only for the manufacture of occluder discs, struts and housing made of metal alloys. The bi-leaflet valve was the first to introduce all PyC on the valve design. Improvements on the PyC is still an ongoing research for surface engineering by coating the material with nanocrystalline titanium oxide and diamond films to improve its contact properties with blood.

Nanomaterials display superior properties and have attracted much attention especially due to their unique high surface to volume ratio which is unavailable in conventional macroscopic materials. The sizes of nanomaterials are similar to most biological molecules and they can be of great use in biomedical applications. In the past few years reports have appeared in the application of metal nanoparticles for the control of microbial infections when used on surfaces of biomedical devices and implants. The antimicrobial properties of both silver and copper nanoparticles have been investigated earlier after coating on to various biomaterials in the last decade (Gallo et al.)
In the last few years, extensive research on metallic nanoparticles has proved their potential as antimicrobial agents. Among these metals the most widely studied metals for their antimicrobial nature are silver and copper (Ingle et al. 2008 & Umer et al. 2012). Copper and silver nanoparticles have gained considerable attention due to their significant and broad spectrum bioactivity. Currently these nanoparticles find utility as antimicrobial formulations, biomedical and surgical devices. Silver ions have been used as an antibacterial component in the coatings of devices employed in medical procedures. Silver and copper in the form of nanoparticles is also of uncoated PyCs, PyC samples coated with thin films of AgNPs and CuNPs to prevent platelet adhesion and thrombosis of the heart valve prosthetic material surface.

The in vitro cytotoxicity evaluation of pure PyC samples, Ag thin films coated PyC and Cu thin films coated PyC samples at 7,500 and 10,000 ablation pulses. This study was used to examine the cytotoxic effects of AgNPs and CuNPs coated PyCs prepared at two different ablation pulses on chick and human cells. Chick fibroblast and human peripheral mononuclear cells (PBMc) were used as model cells for the cytotoxicity studies in order to understand the blood compatibility and immunogenicity of the PyC material coated with metal nanoparticles. The cytotoxic effect of metal nanoparticles was studied by optical microscopy, epifluorescence microscopy and MTT assay. The differences between the responses from the two cell lines to AgNPs and CuNPs coated PyCs were compared.