CHAPTER 5

THE MOST IMPORTANT REQUISITE – \textit{IN VITRO}
HAEMOCOMPATIBILITY OF THIN FILMS OF SILVER
AND COPPER NANOPARTICLES PREPARED BY PULSED
LASER DEPOSITION

5.1 INTRODUCTION

Biocompatibility is the ability of a material to perform with an appropriate host response in a specific application. When a material can be used as a blood interface (in contact with blood) it is called blood-compatible. Blood compatibility is the most important requisite for an implant that interfaces with blood. The implant intended for functioning in contact with blood should not enhance blood clotting or cause damage to the blood components.

Currently used mechanical heart valve prostheses are based on carbon, metals, polymers and their composites. Among these PyC based heart valves are very widely used. Although the mechanical prosthetic heart valves function excellently their usage related problems are found to occur in 30-35\% of patients within 10 years of surgery (Bloomfield et al. 1991). Apart from endocarditis another major problem associated with mechanical heart valve prosthesis made of PyC is platelet aggregation and thrombus formation on its surface. Therefore patients having PyC heart valve replacements require lifelong anticoagulation therapy. But patients who are on anticoagulant treatment for prolonged time are at the risk of bleeding (Baille et al. 1974). It is reported in literature that mechanical prosthetic heart valve thrombosis
(PVT) is a life threatening complication with an incidence of 0.03 to 4% per year (Horstkotte & Burckhardt 1995). The modality of treatment for PVT is surgery with thrombectomy or valve replacement which has a mortality rate varying from 4.7 to 20% (Deveri et al. 1991). Administration of anticoagulants is the major treatment modality for thromboembolic complications till now (Seeburger et al. 2009). For example hemorrhage-bleeding complications occurred at 1% patient per year (Aschermann et al. 2004). Moreover the risk of bleeding from anticoagulant therapy increases with the patient’s age (Cummings et al. 2004; Dvir et al. 2007; Engelmayr et al 2006; Flanagan et al. 2006). Patients above the age of 60 years with mechanical valves and on anticoagulation therapy are nearly seven times more likely to bleed than patients younger than 60 years of age (Zilla et al. 2008; Ajit et al. 2005; Toker et al. 2006).

The increased risk of bleeding in older patients with mechanical valve replacements is the major drawback for the use of these valves (Ye et al. 2009; Harrison et al. 2008). Due to these disadvantages, xenogeneic and allogeneic biological heart valve prostheses have been investigated as an alternative to mechanical heart valve prosthesis. However they also were found to evoke immune reaction, thrombosis and underwent degeneration. Hence while mechanical heart valves have an increased risk of thromboembolism but are more durable, bioprosthetic valves have a decreased risk of thromboembolism but are less durable (Veseley et al. 1989; Walpoth et al. 2007).

When anticoagulation therapy is given to patients with PyC heart valves to combat the problem of thrombosis, there are additional considerations for health care providers and patients. Since warfarin undergoes a variety of interactions with other medications and diet it is difficult to achieve and maintain therapeutically effective levels of warfarin in patients. There is a report that only 62% of patients with mechanical heart
valve replacements and on anticoagulation therapy were found to be within the appropriate international normalized ratio (INR range) in spite of adequate medical adherence (Groves 2001; Massel & Little 2001; Cannegieter et al. 1995). Hence it is essential that patients who are on permanent anticoagulant therapy should make significant and long term lifestyle adaptations such as regular monitoring of INR levels and their diet in order to maintain consistent vitamin K levels, and avoid life threatening complications which result in trauma. Hence it can be said that although mechanical valves offer greater durability it is at the expense of permanent anticoagulation therapy and subsequent higher bleeding risks along with life style modifications to minimize these complications (Gohlke 2000; Oezkan et al. 2000; Pibarot et al. 2000).

In this scenario, it is indispensable to device means to improve the haemocompatibility properties of PyC mechanical heart valves to minimize platelet aggregation and subsequent thrombosis. Only such an approach will obviate the need for lifelong anticoagulation therapy and associated risk of bleeding and other complications. Therefore it was thought that coating of AgNPs and CuNPs as thin films on the mechanical heart valve materials like PyC will be a novel strategy to overcome the hurdle of thrombosis. The coating of AgNPs or CuNPs as thin films on the surface of PyC can be achieved by employing a very simple and yet versatile technique such as PLD.

Silver nanoparticles and copper nanoparticles have found extensive use in biotechnology and biomedical applications in this decade (Spelberg et al. 2008). The specific and favorable properties of silver nanoparticles like antibacterial activity and their nontoxic nature (Krishnaraj et al. 2012; Bakshi et al. 2014) to human cells at low concentrations make them very useful especially in biomedical applications such as wound dressings, contraceptive devices, surgical instruments, bone prosthesis, ocular lens and in cancer
therapy (Cho et al. 2005; Mukherjee et al. 2001; Duran et al. 2005). Similarly copper nanoparticles which are cost effective have wide spectrum antimicrobial activity against various species of microorganisms.

Since rough surfaces promote faster blood coagulation than smooth surfaces it was thought that coating the PyC surface with metal nanoparticles by PLD to bestow a nano smooth surface will mitigate platelet aggregation and thrombosis. Therefore in this work the PyC substrates which were coated with thin films of AgNPs and CuNPs by PLD method were evaluated for their haemocompatibility properties by performing CT, TT, PT, aPTT and anticoagulation assay. The blood compatibility was also determined by SEM for platelet aggregation and thrombus on the biomaterial surface.

### 5.2 RESULTS

An ideal blood compatible material should not activate the intrinsic blood coagulation system or attract platelets. Hence the hemocompatibility of AgNPs and CuNPs coated PyCs was evaluated by CT, TT, PT and aPTT measurements, anti-coagulation assay and platelet adhesion studies.

#### 5.2.1 Haemocompatibility results of thin films of AgNPs coated PyC samples

##### 5.2.1.1 Clotting time test results of films of AgNPs coated PyCs and Control PyC samples

The results of CT measurement are given in figure 5.1. The results showed that metal nanoparticles coated PyC samples had significantly prolonged clotting time than uncoated PyC samples. While PyCs coated at 5,000, 7,500 and 10,000 ablation pulses had significantly higher CT values than uncoated PyCs, the PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 did not behave significantly different from uncoated PyCs. This meant that coating of PyC samples with AgNPs had improved the haemocompatibility properties.
Figure 5.1   Comparison of the CT of control PyC and AgNPs-deposited PyC

5.2.1.2  Thrombin time test results of films of AgNPs coated PyCs and Control PyC samples

The results of TT are given in figure 5.2 which showed that deposition of AgNPs as thin films on PyC samples had improved the TT of PyC substrates. From the results it can be seen that metal nanoparticles deposited PyC samples had significantly higher TT than uncoated PyC samples. PyCs coated at 5,000, 7,500 and 10,000 ablation pulses had demonstrated significantly better haemocompatibility. But PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 did not have significantly higher TT than uncoated PyCs. This demonstrated that PyC samples coated with AgNPs were haemocompatible.
Figure 5.2 Comparison of the TT of control PyC and AgNPs-deposited PyC

5.2.1.3 Prothrombin time test results of films of AgNPs coated PyC samples and Control PyC samples

The results of PT are given in figure 5.3. From the results it can be observed that the deposition of AgNPs as thin films on PyC samples had improved the PT of PyC substrates. From the results it can be observed that metal nanoparticles deposited PyC samples had significantly higher PT than uncoated PyC samples. PyCs coated at 5,000, 7,500 and 10,000 ablation pulses had significantly improved the haemocompatibility. But PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 did not have significantly higher PT than uncoated PyCs.
Figure 5.3  Comparison of the PT of control PyC and AgNPs-deposited PyC

5.2.1.4  Activated partial thromboplastin time test results of films of AgNPs coated PyCsand Control PyCsamples

Figure 5.4 shows the results of aPTT test carried out using uncoated PyCs and AgNPs coated PyCs. When compared to the uncoated PyC samples, AgNPs coated at 7,500 and 10,000 pulses had significantly higher aPTT. However when PyC was coated at lower ablation pulses i.e. 5,000 and at higher ranges i.e. 12,500 and 15,000 pulses, the aPTT time was not significantly different from that of uncoated controls. The results demonstrated that when AgNPs coating was carried out at 7,500 and 10,000 ablation pulses there was improvement in haemocomatibility.

These results demonstrated that coating of both metal NPs at 7,500 and 10,000 ablation pulses were ideal for the improvement of haemocompatibility parameters of the heart valve material PyC.
Figure 5.4  Comparison of the aPTT of control PyC and AgNPs-deposited PyC

5.2.2      Haemocompatibility results of thin films of CuNPs coated PyC samples

5.2.2.1   Clotting time test results of films of CuNPs coated PyCs and Control PyCs samples

Figure 5.5 shows the result of measurement of clotting time test carried out using uncoated PyCs and PyCs coated with CuNPs. Coating of PyC samples with CuNPs improved the haemocompatibility property because those samples had significantly prolonged clotting time than uncoated PyC samples. PyCs coated at 5,000, 7,500 and 10,000 ablation pulses exhibited significantly better haemocompatibility when compared to PyCs coated at 12,500 and 15,000 ablation pulses.
Figure 5.5  Comparison of the CT of control PyC and CuNPs-deposited PyC

5.2.2.2  Thrombin time test results of films of CuNPs coated PyCs and Control PyC samples

The results of TT are given in figure 5.6. From the results it can be observed that the deposition of CuNPs as thin films on PyC samples had improved the TT of PyC substrates. The results showed that the metal nanoparticles deposited PyC samples had significantly higher TT than uncoated PyC samples. PyCs coated at pulses ranging from 5,000 to 10,000 showed significantly improved haemocompatibility property. But PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 did not have significantly higher TT than uncoated PyCs.
Figure 5.6  Comparison of the TT of control PyC and CuNPs-deposited PyC

5.2.2.3  Prothrombin time test results of films of CuNPs coated PyCs and Control PyC samples

Figure 5.7 shows the results of PT test carried out using uncoated and coated CuNPs coated PyCs. It can be observed from the results that CuNPs coating on PyC samples leads to significantly improved PT values when compared to uncoated PyC samples. PyCs coated at 5,000, 7,500 and 10,000 ablation pulses had demonstrated significantly better hemocompatibility than PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 because the latter did not have significantly higher PT values than uncoated PyCs.
5.2.2.4 Activated partial thromboplastin time test results of films of CuAgNPs coated PyCs and Control PyC samples

The results of aPTT are given in figure 5.8. From the results it can be observed that the deposition of CuNPs as thin films on PyC samples had improved the aPTT time of PyCsubstrates. The metal nanoparticles deposited PyC samples had significantly higher aPTT than uncoated PyC samples. PyCs coated at 5,000, 7,500 and 10,000 ablation pulses had demonstrated significantly better hemocompatibility. But PyCs coated at higher ablation pulses i.e. 12,500 and 15,000 did not have significantly higher aPTT than uncoated PyCs.
The results showed that CuNPs coating of PyC substrates at 5000, 7500 and 10,000 ablations made them more blood compatible than uncoated heart valve material.

From the haemocompatibility study it was evident that CT, TT, PT and aPTT parameters of uncoated PyC were significantly lower than that of control blood. But PyC samples coated with AgNPs as well as CuNPs at 5,000, 7,500 and 10,000 ablation pulses had significantly higher CT, TT, PT and aPTT values. This demonstrated that metal nanoparticles coating (with silver or copper)at those ablation pulses conferred the PyC surface with ideal physicochemical properties which improved the blood compatibility of PyC. The PyCs coated at 12,500 and 15,000 ablation pulses did not show significant improvement in the blood compatibility parameters which could

Figure 5.8  Comparison of the aPTT of control PyC and AgNPs-deposited PyC

The results showed that CuNPs coating of PyC substrates at 5000, 7500 and 10,000 ablations made them more blood compatible than uncoated heart valve material.

From the haemocompatibility study it was evident that CT, TT, PT and aPTT parameters of uncoated PyC were significantly lower than that of control blood. But PyC samples coated with AgNPs as well as CuNPs at 5,000, 7,500 and 10,000 ablation pulses had significantly higher CT, TT, PT and aPTT values. This demonstrated that metal nanoparticles coating (with silver or copper)at those ablation pulses conferred the PyC surface with ideal physicochemical properties which improved the blood compatibility of PyC. The PyCs coated at 12,500 and 15,000 ablation pulses did not show significant improvement in the blood compatibility parameters which could
be attributed to their surface roughness which was higher than 45 nm for silver thin films and 28 nm in the case of copper thin films. However the aPTT measurements of AgNPs coated PyCs at 5,000 pulses alone was not significantly higher than the uncoated controls which also can be attributed to the fact that surface roughness when coated at 5,000 pulses was above 35 nm.

5.2.3 Platelet Adhesion studies of thin films of AgNPs and CuNPs on PyC

Platelet adhesion testing was performed to investigate the morphology, adhesion and aggregation of platelets on the surface of AgNPs films coated and CuNPs films coated PyCs at 7500 and 10,000 pulses. These results were compared with those obtained for platelet adhesion test performed using uncoated PyC substrate. Adhesion of platelets on the surface of uncoated PyCs and metal nanoparticles thin films coated PyCs was examined using SEM. Figure 5.9 (a) shows the SEM image of PyC sample after platelet adhesion test was performed on uncoated PyCs. A dense fibrin network with densely aggregated platelets was observed on the surface of uncoated PyCs. This indicated that there was considerable thrombosis on the surface of uncoated PyC controls.

Figure 5.9 (b) and 5.9 (c) show the SEM image of PyC coated with thin film of AgNPs at 7,500 and 10,000 ablation pulses respectively. When compared to control the fibrin network formed on the surface of films coated at 7,500 pulses was less dense and few platelets were found to have adhered randomly on the surface. But on PyC surfaces coated with AgNPs at 10,000 pulses, the fibrin network and platelet adhesion was more when compared to samples prepared at 7,500 pulses. However on the samples coated at 10,000 pulses the density of fibrin network and platelet adhesion was much less than that of uncoated substrates.
Figure 5.9  SEM Morphology of Platelet Adhesion and Aggregation of (A) Uncoated PyC, (B) AgNPs coated PyC at 7,500 ablation pulses and (C) AgNPs coated PyC at 10,000 ablation pulses

Figure 5.9 (d) and 5.9 (e) show the SEM image of PyC coated with thin films of CuNPs deposited at 7,500 and 10,000 ablation pulses respectively. Compared to control PyC samples, PyCs coated with CuNPs both at 7,500 and 10,000 pulses exhibited a fibrin network of similar density on their surfaces. However the aggregation of platelets in the fibrin network was not as dense as that found on uncoated control.

The results of SEM analysis of platelet adhesion on PyCs coated with thin films of metal nanoparticles demonstrated that fibrin network formation and platelet aggregation was less on the surface of samples coated with thin films of AgNPs than that formed on the surface of samples coated with thin films of CuNPs. Also better result in terms of least fibrin network formation and platelet aggregation was demonstrated by AgNPs films deposited at 7500 pulses.
5.2.4 Anticoagulation assay of thin films of AgNPs and CuNPs coated PyC

The anticoagulant effect of uncoated PyCs and thin films of AgNPs and CuNPs coated PyCs by the PLD technique at different ablation pulses ranging from 5,000 to 15,000 was determined by the anticoagulation assay. The clotting time for blood without anticoagulant is normally 5-7 minutes. In the tubes containing uncoated PyC immersed in blood without any anticoagulant at different time intervals i.e. 3 min, 5 min, 7 min, 10 min and 12 min was noted. It was seen that in tubes containing uncoated PyC the blood clot formation was initiated at 5min and clot formation was completed by 7 minutes.
5.2.4.1 The anticoagulant activity of thin films of AgNPs coated PyCs and uncoated PyC samples

The anticoagulant effect of PyC coated with AgNPs at different pulses was evaluated by treating with whole blood without anticoagulant for the time period of 12min. It was very interesting to note that all AgNPs films which were deposited at 5,000, 7,500, 10,000, 12,500 and 15,000 ablation pulses did not cause blood coagulation till 12min. These results clearly demonstrated that PyC surfaces coated with AgNPs thin films inhibited blood clot formation.

5.2.4.2 The anticoagulant activity of thin films of CuNPs coated PyCs and uncoated PyC samples

The anticoagulant effect of PyC coated with CuNPs at different pulses was evaluated by treating with whole blood without anticoagulant for the time period of 12mins. In tubes containing CuNPs films coated at 5,000 and 7,500 ablation pulses blood coagulation was initiated at 10min and was completed by 12 min. In tubes containing CuNPs films coated at 10,000, 12,500 and 15,000 ablation pulses, initiation of blood coagulation was noted at 5min and complete clot formation was observed by 7min. These results showed that among CuNPs films deposited at various ablation pulses, films prepared at 5,000 and 7,500 delayed blood clot formation for a prolonged time i.e. 10 min when compared to films coated at higher ablation pulses.

The evaluation of anticoagulant activity of AgNPs films and CuNPs films demonstrated that between the two metals tested, silver had better anticoagulation activity than copper because all silver films prepared in this study inhibited in vitro blood clot formation. Whereas CuNPs thin films prepared at 5,000 and 7,500 ablation pulses demonstrated considerable anticoagulation activity in that they delayed blood coagulation when compared to uncoated PyCs and normal blood.
5.3 DISCUSSION

Although mechanical heart valves are more durable than biological valves, the former are not without certain disadvantages which are of great concern to the patient as well as health care providers. When blood flows around the mechanical valve at a high shear stress rate it leads to platelet activation and thrombosis on the surface of the valve. Such an event of platelet activation and subsequent thrombosis can lead to embolism. Due to this risk for thrombosis and embolism patients who have mechanical valves are given lifelong anticoagulation therapy. Generally warfarin which is a vitamin K antagonist is the anticoagulant administered to the patients with mechanical heart valves. Although in patients who receive warfarin the risk of thrombosis is reduced, the risk of hemorrhage is increased (Hammermeister et al. 1993; Puvimanasinghe et al. 2001; Oxenham et al. 2003; Van Geldorp et al. 2009; Maggie et al. 2011). It is reported that while a patient with a bio prosthetic valve has a lifetime risk of bleeding of 12% , a similar patient with a mechanical valve replacement has a risk of 41% for bleeding (Rahimtoola 2010; Van Geldorp et al. 2009, Maggie et al. 2011).

It was also found that during anticoagulation therapy the risk of bleeding increases with the increase in the patient’s age (Landefeld & Goldman 1989; Launbjerg et al. 1991; Palareti et al. 1996; Vahanian et al. 2007; Chan et al. 2006). For example patients above the age of 60 years with mechanical valves and on anticoagulation therapy are nearly seven times more prone to bleeding than patients below 60 years of age (Rahimtoola 2010; Maggie et al. 2011). Considering these risks it is preferable to avoid mechanical heart valve replacements in geriatric population. In addition warfarin administration is associated with other considerations for patients and health care providers. Due to barriers to adherence and a variety of interaction between warfarin and diet and other medications, it is very difficult to maintain therapeutically effective levels of warfarin in the body. A
study showed that among patients with mechanical heart valve on anticoagulation drugs only 62% had drug levels within appropriate International normalized ratio (INR) (Rahimtoola 2010; Masters et al. 2001; Maggie et al. 2011).

One approach to obviate the need for anticoagulation therapy and avoid complications like platelet aggregation is to modify the surface of the valve material to enhance blood compatibility. The surface modification of mechanical heart valve in a suitable manner will improve the haemocompatibility and thereby mitigate the problem of platelet activation and thrombosis. Hence in this study efforts were focused on modifying the surface of PyC which is a widely used heart valve material by depositing thin films of nanoparticles of metals namely silver and copper by the application of PLD technique. It was thought that the deposition of thin films of metal particles with dimensions in the nanorange will reduce the platelet activation and aggregation on the biomaterial surface when compared to the uncoated surface of PyC which is relatively rougher. In this chapter the haemocompatibility properties of the heart valve material PyC coated with thin films of AgNPs and thin films of CuNPs were evaluated.

When an artificial material like PyC is brought in contact with the biological system a host of bio physicochemical interactions are initiated at the blood-device interface. In this context the effects on blood and its constituents by the surface modified PyC must be evaluated by assessment of platelet changes, aggregation and thrombus formation. Hence in this study the blood compatibility evaluation involved in vitro tests which can be divided into coagulation time tests and platelet function tests. These included CT, TT, PT, aPTT measurements and anti-coagulation assay along with platelet adhesion and aggregation examination by SEM. Among various metal films prepared those deposited at 7500 and 10,000 ablation pulses were the most effective in inhibiting blood clot formation and silver nanofilms deposited at 7500 pulses were the best in inhibiting platelet aggregation.
Thrombus formation involves the activation of many clotting factors. Hence the hemocompatibility of the surface modified PyC material developed in this study was tested by measuring CT, TT, PT and aPTT. CT is the measurement of the time taken for whole blood to form clot. TT is used to investigate blood clot formation and to evaluate the level and function of fibrinogen. PT test is used to measure the time taken for blood plasma to clot. PT evaluates the coagulation factors II, V, VII, and X of the extrinsic and common pathways of coagulation. The aPTT test is also used to measure the time taken for blood plasma to clot. The aPTT test evaluates factors II, V, VIII, IX, X, XI, and XII of the intrinsic and common pathways of blood coagulation. Since thrombus formation is a multi-step process which involves plasma protein adsorption, platelet adhesion and activation, the AgNPs and CuNPs coated PyC samples were also examined by SEM to assess platelet aggregation on the coated PyCs.

Metal nanoparticles coated PyCs exhibited less platelet aggregation than uncoated PyCs during in vitro platelet rich plasma testing. Especially AgNPs coated PyCs at 7,500 ablation pulses did not exhibit platelet aggregation whereas those coated at 10,000 exhibited only negligible platelet aggregation. On the other hand uncoated PyC samples exhibited in vitro platelet adhesion and aggregation. In the case of uncoated PyC samples the material surface exposed to the blood allowed for greater platelet adhesion and aggregation. But surface coating with metal NPs conferred a nano architecture that was smoother than uncoated surfaces which improved the blood compatibility with subsequent reduction in the extent of platelet adhesion and aggregation.

The biological activity with reference to anticoagulation property of AgNPs thin films and CuNPs thin films deposited on heart valve material PyC was assessed. AgNPs are known to have potential for inhibiting integrin–mediated platelet function responses like aggregation, secretion and adhesion.
to immobilized fibrinogen. There is a report that nano tubes of carbon aggravated platelet aggregation (Debapriya et al. 2012; Radomski et al. 2005). However, very few investigations have been carried out on platelet adhesion with reference to AgNPs. Moreover no report is available with regard to CuNPs. It can be said that the field of study concerning the effect of CuNPs on platelet reactivity is still unexplored.

There is one report that platelet adhesion to immobilized matrices was effectively prevented by nano sized silver particles and intracellular signaling events were also inhibited. In the same study it was demonstrated that nano silver prevented platelet adhesion but without causing lysis and also prevented integrin mediated platelet responses in a concentration dependent manner (Jianget al. 2008; Debapriya et al. 2012). Researchers reported the in vivo anti platelet property of nano silver in a mouse model (Muniyandi et al. 2013; Kemp et al. 2009). These studies showed that even a relatively low dose of nano silver inhibited the function of platelets.

The findings of the present study corroborated with other reports in that the AgNPs and CuNPs coated PyC inhibited platelet adhesion and aggregation when compared to uncoated PyC surfaces. Hence these two metal nanoparticles especially AgNPs can be employed as coatings in the form of thin films on mechanical heart valve surfaces to maintain platelets in a low activation state and prevent thrombosis.

The anticoagulant properties of few engineered nanomaterials have been reported in literature (Ilinskaya & Dobrovolskaia 2013; Krzysztof et al. 2015). Although these nano materials were not intended as anticoagulants it was discovered that they exhibited anticoagulant properties. In vivo testing of perfluorocarbon emulsions as artificial blood substitutes revealed that they had anti-inflammatory and platelet – inhibiting properties. This suggested that they had potential for application in the intervention of ischemia and
thrombosis (Ilinskaya & Dobrovolskaia 2013; Lowe 2000). Some other investigations involving AgNPs showed that PEG coated AgNPs (Ilinskaya & Dobrovolskaia 2013; Ragaseema et al. 2012; Shrivastava et al. 2009) and uncoated AgNPs (Ragaseema et al. 2012; Elbayoumi & Torchilin 2008; Ilinskaya & Dobrovolskaia 2013) also had platelet inhibiting properties.

There is a study in which the anti-coagulative effect of AgNPs of 80 nm particle size was reported. According to these authors no blood clot was formed in the tube containing silver nanoparticles which showed that AgNPs have anticoagulant activity (Muniyandi et al. 2013). In another study the authors reported that gold and silver nanoparticles of about 50 nm inhibited formation of blood clots and thereby exhibited anticoagulant property. Some more reports are available on the biological application of gold and silver nanoparticles based on their in vitro and in vivo anticoagulant activity (Kalishwaralal et al. 2010; Shrivastava et al. 2009). These papers reported the anticoagulant effect of AgNPs which had a mean particle size between 50 – 80 nm. In the present study, thin films of AgNPs and thin films of CuNPs prepared by PLD technique had a particle size of less than 30 nm when compared to those reported by other groups. In this work the mean particle sizes of AgNPs and CuNPs were significantly less than 30nm and this factor can be attributed to their excellent anticoagulant effect.

The blood compatibility of a material is dependent on factors like chemical group distribution, surface texture, smoothness etc. Blood compatibility is the inability of an artificial surface like metal NPs coated PyCs to activate the intrinsic blood coagulation system or attract platelets. Since the AgNPs and CuNPs coated PyCs did not activate the intrinsic blood coagulation system and cause platelet aggregation when compared to uncoated PyCs it can be said that metal NPs coating of PyC surface had vested it with haemocompatibility.
5.4 CONCLUSION

Coating with films of either AgNPs or CuNPs improved the haemocompatibility properties of the PyC surface to a significant extent. CuNPs thin films coated PyCs were more anti-coagulant in nature than uncoated PyC. AgNPs thin films coated PyCs were anticoagulant than those of copper. While coating of PyCs with metal NPs at 12,500 and 15,000 ablation pulses did not show significant improvement in blood compatibility when compared to uncoated PyCs, coating at 7,500 and 10,000 pulses had improved the haemocompatibility of the PyC material significantly. Coating of PyC with silver and copper nanoparticles by PLD at 7500 and 10,000 ablation pulses prevented platelet aggregation and thrombosis on its surface. The surface roughness and particle size of AgNPs films which were below 30 nm and 28 nm respectively and those of CuNPs films which were below 24 nm and 25 nm respectively can be attributed to their blood compatibility. Deposition of thin films either AgNPs or CuNPs by the PLD technique had converted the procoagulant nature of PyC surface to an anticoagulant one.