CHAPTER 6

IN VITRO CYTOTOXICITY OF THIN FILMS OF SILVER AND COPPER NANOPARTICLES PREPARED BY PULSED LASER DEPOSITION

6.1 INTRODUCTION

Certain materials are found to be chemically inert and biocompatible in bulk size exhibit significant toxicity to mammalian cells when prepared in nanoscale (Lewinski et al. 2008, Jeng & Swanson 2006). Nanoparticles can elicit a number of tissue responses such as generation of reactive oxygen species (ROS), cell activation, inflammation and cell death (Johnston et al. 2010; Schrand et al. 2010). The toxicity of AgNPs containing commercial products which are used in medicine must be investigated thoroughly before their application. The application of silver as an antimicrobial agent has been hindered due to its toxic manifestations. It is reported that inappropriate silver deposition in the body will lead to toxic effects such as irreversible pigmentation of the skin and eyes called argyria (Van De et al. 2005). The routes of entry for AgNPs into the human body are the respiratory tract, gastro intestinal tract, skin and through systemic administration (Chen & Schluesener 2008). In addition biomedical implants which contains silver as one of the components or as surface coating material are other ways in which silver can enter the body. Studies on the in vivo oral toxicity of AgNPs showed the accumulation of AgNPs in blood, liver, lungs, kidneys, stomach and brain of Sprague-Dawley (SD) rats. However the oral administration of AgNPs in 60 nm size range for a period of 28 days did not
showed genotoxicity after oral administration at different doses (Kim et al. 2008). Another in vivo study on SD rats revealed that inhalation of AgNPs in particle size range of 1.98 to 64.9 nm did not have any ill effect on the respiratory function, haematology and blood biochemical values (Ji et al. 2007). One group found that a 3D porous graft with PLGA coating composites with 2% of 20-40 nm AgNPs as well as PLGA graft did not have significant in vitro cytotoxicity against pre osteoblast MC3T3-C1 cells. These grafts did not show in vivo toxicity in SD rats during bone regeneration for a period of 8 weeks (Zheng et al. 2010; Liu et al. 2012). Some investigations have shown that AgNPs with an average particle size of 20-25 nm effectively inhibited microbial growth but did not demonstrate significant cytotoxicity (Zheng et al. 2010). Also 10-20 nm AgNPs were found to be non-toxic when administered in vivo by the oral ocular and dermal routes in mice and guinea pigs (Zheng et al. 2010; Maneewattanapinyo et al. 2011). But another toxicity study of 18 nm AgNPs in SD rats reported that prolonged exposure to AgNPs for 90 days had induced alteration in lung function (Sung et al. 2009). It is reported that AgNPs crossed the blood brain barrier and induced neuronal degeneration and necrosis due to accumulation for a long period of time in the brain (Tang et al. 2008). Intravenous administration of 20 as well as 100 nm AgNPs to SD rats suppressed the immune function in a 28 day repeated – dose toxicity studies (Jong et al. 2013; Lee et al. 2007). Early development of fish embryos was affected along with chromosomal aberrations, DNA damage and proliferation in cell lines of zebra fish by AgNPs below 12 nm particle size was reported as findings by one group which indicated that further investigations are essential for elucidating the teratogenic effects in humans (Asharani et al. 2009). There are several studies which have demonstrated the cytotoxic nature of AgNPs to different types of animal cells such as mouse germ cell lines, mouse fibroblast, neuro endocrine cells, and rat liver cells. Similarly AgNPs have been shown to be cytotoxic to various human cell lines such as human alveolar epithelial cell line, human glyoblastoma cells, normal
human lung fibroblast cells and human peripheral blood mononuclear cells (Shin et al. 2007; Hussain et al. 2005; Hsin et al. 2008; Park et al. 2007).

The outcome of above mentioned investigations indicate that conflicting results have been obtained in studies regarding the cytotoxicity of AgNPs against animals and human cells. These conflicting findings make it difficult to attribute the reason for the toxicity of AgNPs to humans. This difficulty arises because the AgNPs employed in various investigations vary tremendously in factors like particle size, and particle aggregation, film thickness in the case of implants. Based on this variation in size, aggregation, thickness etc. of the AgNPs or AgNPs coating, the release profile of silver and its bioactivity will also differ.

The property of large surface area to volume ratio has facilitated the use of CuNPs as a potential antimicrobial agent in many biomedical applications (Avinash et al. 2014). However if any metal nanoparticles is used in excess it might crew toxic to human beings. Therefore extensive investigations on the adverse effects of the use of CuNPs on health have been conducted (Avinash et al. 2014; Galdiero et al. 2011). The in vitro and in vivo toxic effects of CuNPs have not been studied elaborately when compared to silver and gold nanoparticles (Avinash et al. 2014; Valdoker et al. 2011). The toxicity of laser generated cobalt, gold, silver and copper nanoparticles have been studied and compared by Kim et al (2011). The results of this research indicated that the ultrapure nanoparticles showed moderate cytotoxicity to human cells. Although copper is a nutrient required for normal functioning of human body and is maintained in hemostasis, if its intake exceeds the recommended permissible levels it may cause toxic effects like hemolysis, jaundice and subsequently death. Chen et al (2006) have shown that when the intake of CuNPs exceeded the recommended levels due to ingestion or inhalation it exerted toxic effects on the respiratory tract, gastrointestinal tract and some other tissues. They also determined the acute toxicity of micro
copper particles (17 um) and nano copper particles (23.5 nm) in mice and reported that CuNPs were more toxic than copper micro particles due to their easy penetration through skin inhalation and ingestion. Chen et al (2008) also showed that CuNPs caused pathological damage to liver, kidney and spleen. The study by Prabhu et al in which neurons were exposed to CuNPs of increasing concentrations (10-100 ums) and sizes (40, 60 and 80 nm) for 24 hours revealed that there was significant toxic effect with CuNPs of all sizes which were tested when compared to controls which were not treated with CuNPs. According to them nanoparticles with small size and higher concentration exerted the maximum cytotoxicity. It has been shown that PC 12 cell viability decreased with increasing concentrations of CuNPs and treatment time which indicated that decrease of cell viability was directly proportional to concentration and time period of treatment. From these findings it can be understood that the cytotoxicity of CuNPs is size and concentration dependent manner to DRG neurons in rats and PC 12 cells in mice.

Detailed studies are required whenever AgNPs and CuNPs are prepared for a medical application regarding their potential toxicity to humans. When metal NPs coated PyC heart valve prosthesis is implanted in humans there will be intimate contact between the metal NPs and heart tissues and blood flowing through the auricles and ventricles of the heart. This direct contact of body tissues to metal NPs coating on the heart valve material carries the risk for cytotoxicity. This necessitates the need for investigations into the biological effects of silver and copper nanoparticles on human cells.

It is reported in literature that the effect of metal NPs on biological systems is based on their physico chemical characteristics. In addition to chemical composition and the intrinsic toxicological properties of the chemical substance parameters like particle size and shape of the NPs also influence their effect on biological systems. Therefore after performing
physico chemical characterization of metal NPs, toxicological evaluation must carried out to assess the in vitro cytotoxic effects of AgNPs and CuNPs thin films coated PyCs. Hence in this work the cytotoxicity of the metal films coated PyCs on animal and human cells was evaluated.

The aim of this study was to examine the cytotoxic effects of AgNPs and CuNPs coated PyCs prepared at two different ablation pulses on both animal 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.

6.2 RESULTS

6.2.1 Optical microscopy results of silver thin films and copper thin films coated PyC on chick fibroblast and human PBM cells

Optical microscopy study showed that there was a difference in the responses of the two cell types to AgNPs films and CuNPs films. The morphology and number of fibroblast and PBMc population were not affected or reduced after contact with AgNPs films coated with PyC after exposure for 24 hours. Fibroblast and PBM cell populations exposed to AgNPs films prepared at 7,500 as well as 10,000 pulses looked healthy after 24 hours of treatment. The AgNPs films prepared at 7,500 as well as 10,000 pulses were similar in their effect on fibroblast [figure 6.1 (b & c)] and PBM cells [figure 6.3 (b & c)]. Figure 6.1 (a) shows the plates of fibroblast containing uncoated PyCs there were some cells which appeared unhealthy and infected after 24 hours of treatment. In plates of PBMc containing uncoated PyCs [Figure 6.2(a) and 6.4 (a)] there was reduction in the number of cells after 24
hours of exposure. In the case of CuNPs films coated PyC containing plates treated with fibroblast [figure 6.2 (b & c)] and PBM cells [figure 6.4 (b & c)] there was no change in the morphology of the cells but there was a reduction in the density of cells after 24 hours of treatment. This effect was observed with CuNPs films prepared at 7,500 as well as 10,000 ablation pulses against both cell types tested.

Figure 6.1 shows the effect of (A) uncoated PyCs (B) silver thin films prepared at 7,500 pulses and (C) silver thin films prepared at 10,000 pulses on fibroblast cells.
Figure 6.2  Shows the effect of (A) uncoated PyCs (B) copper thin films prepared at 7,500 pulses and (C) copper thin films prepared at 10,000 pulses on fibroblast cells.
Figure 6.3  Shows the effect of (A) uncoated PyCs (B) silver thin films prepared at 7,500 pulses and (C) silver thin films prepared at 10,000 pulses on PBM cells.
Figure 6.4 Shows the effect of (A) uncoated PyCs (B) copper thin films prepared at 7,500 pulses and (C) copper thin films prepared at 10,000 pulses on PBM cells.

As evident from the Inverted optical photo micrographs the AgNPs films coated PyCs did not have any adverse effect on fibroblast and PBM cell populations. Therefore the PyCs coated with AgNPs can be considered as biocompatible material. The results also indicated that although copper was not as cell compatible as silver, nevertheless it did not have any drastic cytotoxic effect. The results also demonstrated that between the two metal NPs coatings silver was more biocompatible to both animal and human cells when compared to copper.
The MTT assay which is a standard cytological test was employed to evaluate the toxicity of AgNPs and CuNPs thin films coated on PyC against chick fibroblast and PBMc. The responses of the two cell types to AgNPs and CuNPs coated PyCs prepared at two different ablation pulses were compared. The difference in the responses of the cell types to the two different metal coatings and the reason behind the difference in responses has been analyzed.

The MTT calorimetric assay involves the determination of ability of viable cells to reduce the soluble yellow soluble Tetrazolium salt [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] [MTT] into a insoluble purple formazon. Tetrazolium salts accept electrons from NADH and NADPH and get reduced. Reduction of MTT takes place in the mitochondrial electron transport chain at the ubiquinone and cytochrome B and C sites by the activity of the enzyme succinate dehydrogenase (Mosmann 1983). The reduction of MTT to formazon inside the cells enables the estimation of mitochondrial metabolism which is an indication of the number of viable cells after exposure to nanoparticles.

The biocompatibility of AgNPs thin films and CuNPs thin films coated on PyCs were evaluated using chick fibroblast and human PBM cells. AgNPs and CuNPs films deposited by PLD at 7,500 and 10,000 pulses were subjected to cytotoxicity tests against the two cell types to determine the appropriate ablation pulse number at which metal NPs should be coated for biocompatible and yet effective antimicrobial activity. In this work the in vitro cytotoxicity of uncoated PyC samples, AgNPs thin film coated PyC samples and CuNPs thin film coated PyC samples were studied. Thin films of AgNPs and CuNPs prepared at 7,500 and 10,000 ablation pulses by PLD on PyC substrates were tested for cytotoxicity and compared.
The cytotoxicities of both metal NPs films were evaluated against chick fibroblast cells as well as human cells namely PBM cells. The cytotoxic effects of the two metal NPs along with uncoated PyCs on the viability of fibroblast and PBM cells were analyzed using MTT assay in six well plates in MEM and RPMI medium respectively. Untreated fibroblast and PBM cells served as negative controls. Results were quantified as relative values to that of negative control (untreated cells) which was set as 100% viability.

6.2.2 Cytotoxicity assessment of uncoated PyC samples against chick fibroblast and human PBM cells

From the results it was evident that uncoated PyC samples were cytotoxic to both chick fibroblast cells and PBM cells because it reduced the percentage of viable cells when compared to untreated plates. This showed that PyC material by itself had cytotoxic effect on both cell types tested in this work.

6.2.3 Cytotoxicity assessment of silver thin films coated on PyC against fibroblast cells by MTT assay

The results of cytotoxicological testing of PyCs coated with AgNPs against chick fibroblast showed statistically comparable live cell percentage values to negative control for Ag thin films coated at 7,500 as well as 10,000 ablation pulses. These results demonstrated that AgNPs films deposited at both ablation pulses had no cytotoxic effect on chick fibroblast and were totally biocompatible.
Figure 6.5  Show the cytotoxic effect of uncoated PyCs, silver thin films prepared at 7,500 and 10,000 ablation pulses against chick fibroblast cells

6.2.4  Cytotoxicity assessment of silver thin films coated on PyC against PBM cells by MTT assay

Uncoated PyC samples showed cell viability which was significantly lower than that of negative control when tested against PBM cells. AgNPs coated at 7,500 pulses maintained a live cell percentage that was statistically near comparable to that of negative control and significantly higher than that of untreated PyCs. This very clearly indicated that AgNPs films coated at 7,500 pulses was not cytotoxic to PBM cells. The AgNPs coated PyC films at 10,000 pulses also showed a live cell percentage that was less but comparable to untreated negative control. However it was significantly higher than that of uncoated PyC treated cells. This indicated that AgNPs films coated at 10,000 pulses were also not cytotoxic to PBM cells. Between the AgNPs films coated at 7,500 and 10,000 ablation pulses, those coated at 7,500 pulses showed better cytocompatibility.
Figure 6.6  Show the cytotoxic effect of uncoated PyCs, silver thin films prepared at 7,500 and 10,000 ablation pulses against PBM cells

6.2.5  Cytotoxicity assessment of copper thin films coated on PyC against chick fibroblast cells by MTT assay

The results of the cytotoxicity tests of CuNPs films coated PyCs revealed that those coated at 10,000 pulses were non cytotoxic as they had a live cell cell percentage which was near comparable to that of untreated negative control and statistically higher than that of uncoated PyC samples. The live cell percentage values of CuNPs films prepared at 7,500 ablation pulses was lower than untreated controls and similar to uncoated PyC treated fibroblast cells. These results demonstrated that CuNPs coating was not cytotoxic to fibroblast cells when prepared at 7,500 as well as 10,000 ablation pulses although the films deposited at 10,000 pulses were more biocompatible than those deposited at 7,500 pulses.
Figure 6.7 Show the cytotoxic effect of uncoated PyCs, copper thin films prepared at 7,500 and 10,000 ablation pulses against fibroblast cells

6.2.6 Cytotoxicity assessment of copper thin films coated on PyC against PBM cells by MTT assay

The plates treated with CuNPs coated PyC films prepared at 7,500 pulses and 10,000 showed a live cell percentage greater than the uncoated negative controls. These CuNPs films deposited at both ablation pulses were not cytotoxic to PBM cells. The results also showed that CuNPs films prepared at 7,500 were less cytotoxic when compared to uncoated PyC samples. CuNPs at 10,000 pulses were had similar cytotoxic effect like uncoated PyCs which showed that coating at 10,000 pulses had not improved the significantly more biocompatible than uncoated PyC samples. Between the two types of CuNPs films the films deposited at 7,500 pulses showed less cytotoxic effect on PBM cells than 10,000 pulses.
Figure 6.8  Show the cytotoxic effect of uncoated PyCs, copper thin films prepared at 7,500 and 10,000 ablation pulses against PBM cells

This study indicated that AgNPs and CuNPs prepared at both the ablation pulses showed minimal cytotoxicity against both animal and human cells. The cytotoxic effect of CuNPs was noted to be greater than that of AgNPs on both fibroblast and PBM cells.

6.3 LIVE AND DEAD CELL STAINING BY EPIFLUORESCENCE MICROSCOPY

The extent of cell death of chick fibroblast and human PBM cells caused due to contact with metal NPs coatings on PyC surfaces was studied by Epiflourescence microscopy after performing the live/dead cell assay.
6.3.1 Live and dead cell assay of silver thin films coated PyC against fibroblast cells

Figure 6.9 (a) is the epifluorescent micrographs of untreated chick fibroblast cells. Figure 6.9 (b), (c) and (d) are micrographs of uncoated PyC, AgNPs coated at 7,500 and 10,000 ablations respectively after treatment with cells. In plates containing fibroblast treated with uncoated PyCs the number of viable cells had reduced when compared to untreated cells. In plate containing AgNPs coated PyCs at 7,500 and 10,000 ablation pulses, the number of viable cells was more when compared to uncoated PyCs. This indicated that AgNPs coating of PyCs was not cytotoxic to chick fibroblast and had improved the biocompatibility of the heart valve material.

Figure 6.9 Shows the cytotoxic effect of (A) untreated cells (B) uncoated PyCs (C) silver thin films prepared at 7,500 pulses and (D) silver thin films prepared at 10,000 pulses on fibroblast cells
6.3.2 Live and dead cell assay of silver thin films coated PyC against PBM cells

Figure 6.10(a) is the epifluorescent micrographs of untreated human PBM cells. Figure 6.10 (b), (c) and (d) are micrographs of uncoated PyC, AgNPs coated at 7,500 and 10,000 ablations respectively after treatment with cells. When compared to untreated cells, there was more number of dead cells in the plates containing uncoated PyCs. The results clearly showed that a very high amount of live cells and negligible amount of dead cells were present in plates containing PyCs coated with AgNPs at 7,500 and 10,000 ablation pulses.

Figure 6.10 Shows the cytotoxic effect of (A) untreated cells (B) uncoated PyCs (C) silver thin films prepared at 7,500 pulses and (D) silver thin films prepared at 10,000 pulses on PBM cells
6.3.3 Live and dead cell assay of copper thin films coated PyC against fibroblast cells

Figure 6.11 (a) is the epifluorescence micrographs of untreated chick fibroblast cells. Figure 6.11 (b), (c) and (d) are micrographs of uncoated PyC, CuNPs coated at 7,500 and 10,000 ablations respectively after treatment with cells. In plates containing fibroblast treated with uncoated PyCs the number of viable cells had reduced and some dead cells were present when compared to untreated cells. The plates containing CuNPs coated PyCs at 7,500 pulses had a viable cell density similar to that of uncoated PyCs. Whereas the CuNPs coated PyCs at 10,000 ablation pulses, the amount of viable cells was more when compared to uncoated PyCs and CuNPs coated PyCs at 7,500 ablation pulses. This indicated that CuNPs coating of PyCs at 10,000 ablations rendered the heart valve PyC material more compatible to animal cells.

Figure 6.11 Shows the cytotoxic effect of (A) untreated cells (B) uncoated PyCs (C) copper thin films prepared at 7,500 pulses and (D) copper thin films prepared at 10,000 pulses on fibroblast cells
6.3.4 Live and dead cell assay of copper thin films coated PyC against PBM cells

Figure 6.12 (a) is the epifluorescent micrographs of untreated human PBM cells. Figure 6.12 (b), (c) and (d) are micrographs of uncoated PyC, CuNPs coated at 7,500 and 10,000 ablations respectively after treatment with cells. In the plates containing uncoated PyCs, the number of live cells was reduced and dead cells were also present when compared to untreated cells. In the case of CuNPs coated PyCs at 7,500 as well as 10,000 pulses, the plates contained both viable and dead cells similar to uncoated PyCs. This indicated that CuNPs prepared at 7,500 and 10,000 pulses were similar effect on the viability of PBM cells.

Figure 6.12 Shows the cytotoxic effect of (A) untreated cells (B) uncoated PyCs (C) copper thin films prepared at 7,500 pulses and (D) copper thin films prepared at 10,000 pulses on PBM cells
6.4 DISCUSSION

In the past few decades there has been rapid development in the field of nanotechnology which involves preparation, characterization and manipulation of artificial structures whose features are in the nanometer range (Chaloupka et al. 2010; Alt et al. 2004). This empowers researchers to prepare nanoparticles and create products by incorporating them into materials and thereby confer novel properties to the products (Zheng et al. 2010; Nadworny et al. 2008). There is a surge of interest in the application of nanoparticles for biological purposes in recent years. Hence the use of nanomaterials as antimicrobial agents, biosensors, drug carriers and coating of surfaces of biomedical devices like stents, catheters and cardiovascular implants has been growing rapidly. In biology, the application of metal NPs like Ag and Cu have been widely studied with special reference to their antimicrobial properties (Pulit et al. 2013; Van de et al. 2001; Xu et al. 2012; Yoon et al. 2007).

The development in fronts like preparation of materials in nanoscale by techniques like PLD has led to an increase in the surface-to-volume ratio of AgNPs and consequently their antibacterial activity. However while the commendable antibacterial activity of AgNPs has resulted in their wide application in medical devices and many other products, their toxic effects on human cells have resulted in the need for regulatory measures to contain the prolific use of nanoparticles (Martinez et al. 2010; Esteban et al. 2006).

AgNPs have been reported to have cytotoxic effects on both normal and cancer cells in mammals (Asharani et al. 2009; Shin et al. 2007; Hsin et al. 2008). The modes of interactions of AgNPs have also been studied in various prokaryotic and eukaryotic organisms (Carlson et al. 2008; Jeong et al. 2005). Reports are found in literature about the cytotoxicity of silver ions
in different cell lines as well (Ghanbari et al. 2009; De Mel et al. 2012; Van De et al. 2005; Vik et al. 1985). Cytotoxic effect of silver nanomaterials on microbes is wanted; great concern has also emerged about the safety issue of these nanoparticles because of the undesirable adverse effects of AgNPs on the environment and the health of humans (Van De et al. 2005; Mandal et al. 2006).

Like silver, copper is also used in the nanoparticulate form due to its large surface area-to-volume ratio and finds use as antimicrobial agents in several biomedical applications. Since the excess use of any metal NPs is toxic to living beings and environment. The CuNPs have also been scrutinized for their cytotoxicity. Although studies on bioactivities of CuNPs have proved their effectiness against pathogenic bacteria, fungi, algae and viruses the use of CuNPs at high concentrations and beyond the safety limits leads to adverse reactions and serious cytotoxicity issues.

While the antimicrobial properties exhibited by metal NPs is desirable their cytotoxic effects on animal and human cells is precarious to health and life. Hence it is mandatory that nanomaterials intended for human application should be subjected to cytotoxic studies to assess its potential effects on the human system. The application of AgNPs and CuNPs coated PyC heart valves will result in the potential exposure of patients having the biomedical implant to silver and copper nanoparticles. Therefore in this work the cytotoxic effect of thin films of AgNPs and thin films of CuNPs coated PyCs by the PLD technique was studied to assess its biocompatibility to cells. The thin films of both AgNPs and CuNPs prepared at 7,500 and 10,000 were tested against chick fibroblast cells and human PBM cells.

The MTT assay is a very sensitive assay for the determination of cytotoxicity of substances and materials. In the MTT assay the ability of the mitochondria of viable cells to reduce the soluble, yellow MTT into an
insoluble purple formazon is estimated. Hence the reduction of MTT to formazon indicates the decrease in mitochondrial metabolism of the cells. So the mitochondrial metabolism of cells decreases reduction of MTT to formazon increases. Hence the amount of formazon formed can be correlated with number of cells with intact mitochondrial metabolism after exposure to metal nanoparticles.

It was interesting to note that heart valve material PyC was by itself cytotoxic to cells. Coating of PyC with AgNPs had improved the biocompatibility of PyC to a significant extent. Coating at both pulses i.e. 7,500 and 10,000 had conferred excellent biocompatibility to PyC. It is very obvious from this study that if AgNPs are deposited as thin films on PyC surface by PLD technique at 7,500 and 10,000 ablation pulses, it will prevent microbial infection and thrombosis without any compromise on biocompatibility issue. Coating of PyC with CuNPs did not significantly improve the biocompatibility of PyC when compared to negative controls. However coating of PyC at 7,500 pulses improved the biocompatibility towards PBM cells, whereas coating at 10,000 pulses improved the biocompatibility towards fibroblast cells. Therefore coating of thin films of AgNPs and CuNPs by PLD on heart valve material PyC at 7,500 and 10,000 ablation pulses can be considered as a safe approach for inhibiting microbial invasion and platelet adhesion.

Very few have reported earlier on the surface modification of mechanical heart valve material PyC using metals such as silver and copper. Tang et al (2007) have reported that Ag⁺ - implanted PyC samples did not show cytotoxicity and had good biocompatibility. But in this work Ag was employed in PyC in ionic form (Ag+) and not in the nanoparticulate form. Moreover in this silver ion was implanted in PyC but not deposited as a nanoparticulate thin film coating on the PyC surface. Although the in vitro and in vivo cytotoxic effect of silver and copper NPs is well documented in
literature, the cytotoxic effect of AgNPs and CuNPs when deposited as coatings on PyC is not known. This work is first of its kind to report the results of cytotoxicity testing of AgNPs and CuNPs deposited as thin films coating on the heart valve material PyC.

Liu et al (2007) who have worked with coating of Cu layer on PyC and reported their antibacterial effect have not studied their cytotoxic effect and biocompatibility. Moreover their work did not employ Cu in the nanoparticulate form on PyC surface.

The AgNPs and CuNPs coated PyCs which were toxic to bacteria and inhibited their growth did not show toxicity to animal and human cells. There is a work which reported that eukaryotic cells require a higher concentration of AgNPs to exert cytotoxic effects than prokaryotic cells. Hence Ag nanomaterials are cytotoxic to humans only at higher concentrations than those required to induce toxicity on bacteria. This could be due to the fact that eukaryotic cells are more complex in size, structure and biological function than prokaryotes (Masse et al. 2010; Yoon et al. 2007; Pulit et al. 2013). The results of this work corroborated in that AgNPs and CuNPs films prepared by PLD on PyC heart valve prosthetic material were effective in killing bacterial cells without showing cytotoxicity in animal and human cells.

6.5 CONCLUSION

The in vitro cytotoxicity evaluation of Ag thin films and Cu thin films coated PyC samples at 7,500 and 10,000 ablation pulses was studied against chick fibroblast and human PBM cells by MTT assay, optical microscopy and epifluorescence microscopy and compared with uncoated controls. AgNPs films coated at both 7,500 and 10,000 pulses were not cytotoxic to fibroblast as well as PBM cells with coating at 7,500 pulses being more biocompatible. While CuNPs films coated at 7,500 pulses was more
compatible than 10,000 pulses against PBM cells whereas against fibroblast cells CuNPs coated at 7,500 pulses was less compatible when compared to coating at 10,000 pulses. Optical and epifluorescence microscopy revealed that AgNPs coating of PyC did not adversely affect the morphology and number of cells in the populations of both tested cell lines. Between the two metals silver was more biocompatible to both cells than copper in the nanoparticulate form.