QATAR UNIVERSITY

COLLEGE OF ARTS AND SCIENCES

PREPARATION AND CHARACTERIZATION OF MULTILAYERED ELECTROSPUN COMPOSITE SCAFFOLDS FOR BIOMEDICAL APPLICATIONS.

BY

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ABSTRACT

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Title: Preparation and Characterization of multilayered electrospun composite scaffolds for biomedical applications.

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Electrospinning has gained wide attention recently in biomedical applications. Electrospun biocompatible scaffolds are well-known for biomedical applications such as drug delivery, wound dressing and tissue engineering applications. In this study, composites based electrospun polymer fibers Polycaprolactone (PCL) and polyvinyl alcohol (PVA) was produced by using electrospinning technique in which multilayered structure (PCL-PVA-PCL) loaded with gentamicin sulphate (GS) (drug) in the middle layer with PVA. Formerly, metal silver particles were deposited on the surface of electrospun fibers using plasma sputtering technology. The electrospun scaffolds were characterized by scanning electron microscope (SEM), Energy Dispersive X-ray Spectroscopy (EDX), Fourier Transform Infrared Spectroscopy (FTIR), Water contact angle measurement, X-ray Diffraction (XRD) and Thermogravimetric Analysis (TGA). The drug delivery of gentamicin sulphate from the multilayered electrospun PCL-PVA-PCL was investigated by using calorimetric method. The outcomes prove that, initial burst release of the drug could be reduced with the increase in the drug loading from 1% to 4%GS in the multilayered structure. Furthermore, the antibacterial properties of the sample were examined. The multilayered electrospun fiber loaded with drug and sputter
coated with Ag has enhanced the antibacterial efficiency from the control. In addition, biological performance such as cell cytotoxicity was investigated on the cell line fibroblast (Wi38) and human keratinocyte (HaCaT) by using alamar blue assay. The presence of Ag has revealed certain toxic effect with both cell line, and also the increase in the gentamicin concentration has also exhibited particular toxicity. Finally, In-vitro release of silver from the surface of the scaffold was inspected. The results show a uniform release of silver from the surface rather than initial burst release. Therefore, the formulated scaffolds are suitable candidate for biomedical application such as wound healing for prolonged antibacterial inhibition through uniform release of drug as well as silver particles from the surface of the scaffolds.
I dedicate this work to my better half, Shajil Kareem for being a good supporter and strength provider in my life. In addition, to my son Ewaan Wildan and my upcoming baby (In Sha Allaah).

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INTRODUCTION

Electrospinning is a technique used for the preparation of polymer Nano-micro fibers which have great attention in the biomedical industry. The fibers prepared by electrospinning process have high-surface volume ratio, adjustable porosity, tailored composition and properties. Therefore, it can electrospun wide varieties of polymers such as natural polymers, synthetic polymers and biodegradable polymers. These micro/nano fibrous polymers have several advantages such as the fiber scaffold mimics extracellular matrix. Therefore, it enhances the cell adhesion, proliferation, migration and differentiation; the scaffolds show higher volume to surface ration, higher porosity with customized pore size. All this opens for the release of biofactors such as drug, protein, genes as well as promote the nutrients and oxygen diffusion plus waste removal. In addition, morphology of electrospun nanofibers such as core-shell, hollow, nanowire-microtubers and three-dimensional fiber scaffold could be modified by changing the parameters of electrospinning process. Thus, these precious factors make the electrospun nanofibers suitable for biomedical applications such as drug delivery, tissue engineering and wound healing.

Drug delivery idea emerged in the 1970’s for the controlled release of drug for therapeutic treatments [1]. The high surface area and porosity of polymer fibers grabbed great attention in recent years to use as a drug carrier. The polymer fibers morphologies and bulk properties can be modified by the use of electrospinning technique. In this process, polymer nanofibers loaded with drugs are synthesized for drug delivery. Drugs ranging from antibiotic and anticancer agents to proteins, aptamer, DNA[2] and RNA[3]
have been incorporated into the nanofiber. The release mechanism of drugs in polymer fiber can be altered by the change in the type of drug loadings, such as co-axial electrospinning, emulsion electrospinning, multiple layers, blended electrospinning and co electrospinning etc. However, multi-layered electrospun fibers have shown a great application in drug delivery due to the sustained release of the drug rather than initial burst release of drug from the fiber scaffolds. Innovative design for the controlled release of drug is layer-by-layer nanofibers stacking sandwich with drug loaded in between. This type of design is very simple, easily controllable, and readily fabrication process as compared to core/shell. The drug release mechanism of multilayers fibrous mats could be controlled by adjustment in the thickness of the outer layer, amount of drug loaded, porosity of the scaffolds etc. One of the methods for regulating the delivery of drugs from an electrospun fiber mats loaded with drug system and altering the release behavior over a specific time is to use hydrophilic and hydrophobic polymers [4].

PVA known as polyvinyl alcohol is a semicrystalline hydrophilic polymer which is easily soluble in water. The solubility in water makes the PVA a great application in drug delivery. PVA is a biocompatible, biodegradable and easily electrospinnable polymer. However, individual PVA cannot be used for drug delivery due to its water solubility. As it will lead to burst release of the drug. PVA is fused with chitson to improve its biocompatibility and cell attachment [5][6]. On the other hand, PCL, known as polycaprolactone, is a hydrophobic polyester polymer widely studied in electrospinning. PCL has a great biomedical application due to its biocompatibility, biodegradation, mechanical property, non-toxicity, low cost and low melting point. PCL properties like biodegradability, cytotoxicity and degradation rate were studied elaborately for short and
long duration in implantations [7], [8]. Degradation of PCL is non-enzymatic which means by hydrolysis. PCL fibers are well studied in as a drug carrier in drug delivery. In a study, the release of model drug tetracycline hydrochloride (TC-HCL) and phenytoin sodium from the PVA-PCL-PVA electrospun nanofibers was reported. The hydrophilic and hydrophobic polymer was prepared layer by layer by incorporating multiple drugs such as PHT-Na to OVA and TC-HCL to PCL, respectively.

Other than drug delivery, electrospinning techniques has gained much consideration in tissue engineering. The electrospun fibers structure possesses many characters suitable for tissue engineering such as mechanical properties, high surface to volume ratio and adjustable porosity. Tissue engineering is an advance field of science which merge with applied engineering and bioscience for constructing biomaterials that recover, sustain or improve the biological activities of injured tissues [9]. Wound healing or skin tissue engineering has been studied very well in recent years. The wound healing process takes place in four different steps: hemostasis, inflammation, proliferation and remodeling [10]. The wound healing is stabilized by different cells, growth factors and cytokines. In the first process, the host cells and bacteria are removed by the macrophages in the inflammation step. These macrophages also secret some factors eligible for the angiogenesis, fibroplasia and extracellular matrix production [11]. The endothelial cells proliferation, migration and remodeling are important factors that contribute to the angiogenesis [40]. Finally, fibroblast proliferation leads to the rebuilding of the function and structure in the injured site[13]. During these all stages, antibacterial protection is an important factor. In electrospun fiber antibacterial agents such as antibiotics and silver are incorporated. Silver is a transition metal in the periodic table. The silver related
compounds or nanoparticles have a biocidal effect on around 16 specious bacterial because of its toxic effect on microorganisms [14][15]. The silver nanoparticles were added to the electrospun fibers via Ag ions through the wetting process[16][17], silver sulfadiazine [18], etc. The wetting process for the preparation Ag to the matrix have many disadvantages such as uneven distribution of nanoparticles, using reducing agents that are toxic, and controlling the size of nanoparticles is a difficult task depending on the strong and weak reducing agents used[19]. However, the most efficient way of introducing nanoparticles to the surface of polymer or fabrics could be done by using plasma technology. Plasma technology provides a uniform deposition, less use of resources as well as a simple process for the coating of an antibacterial material such as Ag, Si, Cu etc, to the surface of the polymer than wetting process.

In this work, drug delivery mechanism of multilayered electrospun fiber mats of PVA and PCL loaded with Gentamicin sulfate sputter coated with silver was studied. As well as cell toxicity of the fiber mats was studied. Simultaneously, sputter coated silver nanoparticles on the PCL electrospun fiber was studied. And we investigate the effect of cytotoxicity of silver with the human cell line (HaCaat and Wi38). Finally, in-vitro release of silver from the fiber scaffold was studied for investigating the longevity of antibacterial property of the material. Antibacterial effect was also demonstrated in this work for wound dressing applications.

1.1 Objectives

- To optimize the individual fibers of PCL, PVA and multilayer electrospun fiber mats.
• To prepare electrospun fiber mats in multilayer (PCL-PVA-PCL) design loaded with gentamicin sulfate to investigate the effect on drug release behavior on scaffold loaded with drug at different percentage

• To investigate the release pattern of Ag from the composite scaffold for longevity of antibacterial properties.

• To investigate the cytotoxicity of the prepared fiber mats for biomedical applications.

• To introduce sputter coating technology to the fiber mats for antibacterial activity.

• Study the cytotoxicity of the prepared scaffolds.

• To investigate the antibacterial property of the prepared fiber mats
LITERATURE REVIEW

1.2 Introduction:

In the last 20 years, emergence of nanotechnology has gained much attention for electrospinning process. This process is used for the preparation of polymer Nano-micro fibers and it has great importance in biomedical industry due to its cost effectiveness, scalability, versatility and simplicity. The process was invented in 1901 by JF Cooley and WJ Morton but had slower development over 100 years. Later in the years, Reneker [20] invented the fiber preparation from organic polymer, which created a new field of science for the formulation of fibers ranging between nm – µm.

Electrospinning device includes four main components Figure (1) high power supply, syringe pump, syringe needle with solutions and a collector for fiber deposition. The electric field passes through the polymer solution, where the positive electrode is connected to the needle and the negative electrode to the collector. Hence, when the voltage is applied, the repulsive charge accumulates at the tip of the needle which is shaped in the form of a hemisphere[21]. When the electric filed is applied the solution at the tip of the needle should possess high surface energy and surface tension. When the repulsive charge overcomes the surface tension, it leads to the formation of Taylor cone. This will direct the polymer solution towards the negative electrode as collector and produce fibers. The solvent from the polymer solution is evaporated and the polymer solution is deposited on the collector as dry fibers ranging from nanometers to micrometers[22].
The fibers prepared by electrospinning process have high-surface volume ratio, adjustable porosity, tailored composition and properties. Therefore, it can electrospin wide varieties of polymers such as natural polymers, synthetic polymers and biodegradable polymers. These micro/nano fibrous polymers have several advantages such as the fiber scaffold mimics extracellular matrix. Therefore, it enhances the cell adhesion, proliferation, migration and differentiation; the scaffolds show higher volume to surface ration, higher porosity with customized pore size. All this opens for the release of biofactors such as drug, protein, genes as well as promote the nutrients and oxygen diffusion plus waste removal. In addition, morphology of electrospun nanofibers such as core-shell, hollow, nanowire-microtubers and three dimensional fiber scaffold, could be
modified by changing the parameters of electrospinning process. Thus, these precious factors make the electrospun nanofibers suitable for biomedical applications such as drug delivery, tissue engineering and wound healing.

1.3 Drug delivery

Drug delivery idea emerged in the 1970’s for the controlled release of drug for therapeutic treatments [1]. The high surface area and porosity of polymer fibers grabbed great attention in recent years to use as drug carrier. The polymer fibers morphologies and bulk properties can be modified by the use of electrospinning technique. In this process, polymer nanofibers loaded with drugs are synthesized for drug delivery. Drugs ranging from antibiotic and anti-cancer agents to proteins, aptamer, DNA[2] and RNA[3] have been incorporated into nanofiber. The release mechanism of drugs in polymer fiber can be altered by the change in the type of drug loading, such as co-axial electrospinning, emulsion electrospinning, multiple layer, blended electrospinning and co electrospinning etc. However, co-axial electrospinning and multi layered electrospun fibers have shown great application in drug delivery due to the sustained release of the drug rather than initial burst release of drug from the fiber scaffolds. Therefore, recent advancement in the field of electrospinning for drug delivery will be discussed in the proceeding paragraphs. Electrospinning in drug delivery is categorized into the drug loading type, drug loading materials and type of drugs.

1.4 Drug loading type

The electrospinning have different drug loading type which determines the diverse structure and different drug release kinetics. The drug loading procedure in
electrospinning could be executed in different ways such as co-electrospinning, multi-electrospinning, side by side electrospinning, co-axial, surface immobilization and emulsion electrospinning Figure (2). The co-electrospinning drug molecules are mixed with the polymer solution prior to electrospinning. These electrospun fibers provide uniform distribution of drugs/biomolecules and high drug/biomolecules loading. However, the biomolecules properties could be damaged when they face the high voltage directly. On the other hand, blend electrospinning or side by side electrospinning help to sort the issue with the drug and molecules solubility in common solvents. Moreover, the multi-jets with more than two spinnerets exhibit a way to protection for the bioactivity of drug. Furthermore, surface immobilization is another method where drug molecules are covalently bonded with the scaffolds via chemical or physical immobilization method. In this chemical methods, the surface of nanofibers is changed by introducing amines, carboxyl, hydroxyl or thiol; the physical method includes the incorporation of vaderwaals, electrostatics and hydrophobic interactions. This immobilization retains the biomolecule activity, but all the electrospinning process show burst release kinetics of the drug molecules. In this situation, co-axial and emulsion electrospinning process are introduced. In co-axial and emulsion electrospinning is where the biomolecules are incorporated with the core protected by shell molecules from environmental defects. They also provide sustained release of drug by minimizing initial burst release by controlling the thickness and composition of the shells. Another advantage of drug loading for sustained release of drug is layer by layer via addition of drug in between the electrospun scaffolds; this controlled release is promoted by the shield provided by the layer polymer scaffolds. Therefore, co-axial electrospinning and multilayer
electrospinning drug loading type provide a better sustained and controlled release of drug.

Figure 2: Schematic representation of different type of electrospinning process[23].
1.4.1.1 **Multiple layered fiber mats.**

Multilayered fiber mats provide the control release of drug in a layer-by-layer nanofibers stacking sandwich with drug loaded in between. This type of design is very simple, easily controllable, and readily fabrication process as compared to core/shell. The drug release mechanism of multilayers fibrous mats could be controlled by adjusting the thickness of the outer layer, the amount of drug loaded, porosity of the scaffolds etc. The designing of core/shell structure is a difficult process in one sense due to the diverse properties of electrical and rheological properties such as conductivity and surface tension of the core and shell polymer materials [24]. Hence, due to difficulty in fabricating core/shell, electrospun fiber mats could not achieve a sustained repeatability, and also controlled release of drug from the structure is difficult to study efficiently. There are many fibers incorporated with drug molecules that were electrospun with varied thickness and studied its drug delivery mechanism. Geun Hyung Kim [25] prepared PCL-PEO-PCL layered fiber mats and drug delivery was studied with various thickness of PCL outer layer. It is shown that, burst release can be erased by increasing the thickness of the PCL layer as well as by incorporating antimicrobial peptide HPA3NT3 which doesn’t lose its biological activities. On the other hand, sustained release of drug haloperidol was investigated by changing the hydrophobicity of the scaffolds. Herein, PVA-methylated b-cyclodextrin was incorporated with PLA and PLGA. Addition of b-cyclodextrin reduces the fiber degradation rate of PVA [26]. It is noted that, as the hydrophobicity of the scaffold increases the release of hydrophilic drug is sustained in a controlled manner. Where, the polyester polymers release the drug via hydrolysis manner. The blending of hydrophobic and hydrophilic drug will minimize the toxicity caused by the burst release
of drug. This type of combination can be applied in hydrophobic and heat sensitive drug due to the simplicity of the process.

The drug delivery of ibuprofen from sandwich layered fibers mates was studied and its mathematical modeling was elaborated by using power law, higuchi equation and Fick’s second law was applied [27]. The mathematical modeling suggests that, thickness of the fiber mats concern more on drug delivery than the concentration of the loaded drug. Here, PLA was electrospun successfully by incorporating ibuprofen drug in between the two layers of PLA. Finally, according to the type of treatment the drug loading can be changed in accordance with the thickness of the layers for the controlled release of drug. Dave Wei-Chih Chen [28] has studied the drug delivery of vancomycin, gentamicin, and lidocaine for wound healing applications. Here in, they successfully mixed PLGA/collagen on the outer layer and PLGA loaded with drug in the middle layer. The drug vancomycine and gentamicin has released in high concentration from biodegradable polymer scaffold. However, lidocaine has shown release upto 3 weeks. The bioactivity of drug was shown 40-100% efficiency and it was concluded that this scaffold was suitable for boosting the wound healing process at initial stage of wound.

1.5 Drug loading materials

Varieties of polymers can be electrospun into diverse design for drug delivery applications in accordance with polymer-drug compatibility and ability to be molded to fit for variety of delivery routes. While designing an optimized drug delivery system, there are many polymer factors to be considered. For instance, biocompatibility, biodegradability, mechanical properties and hydrophilicity [29]. There are many polymer varieties such as natural and synthetic polymers that are used for designing drug delivery
A diverse range of drug have been loaded into the system such as growth factors, DNA, proteins, inhibitors and antibiotics [30][31][32].

Electrospinning process can be easily applied for synthetic polymers with great flexibility. However, synthetic polymers affect cell affinity due to the hydrophobic nature and smooth surface for the cell recognition sites. On the other hand, natural polymers show enhanced biocompatibility, some exhibit antibacterial properties and better clinical functionality. The natural polymer include cellulose, chitosan, chitin, dextrose, collagen, silk, gelatin etc [33]. For instance, lee et al. concluded the features of diverse polysaccharide on electrospinning and their biomedical applications such as drug delivery, wound dressings and enzyme immobilization[34]. The studied polysaccharides are such as cellulose, chitosan, alginate, chitin, starch, hyaluronic acid, dextran and heparin. Chitosan polymer had the properties of anticancer due to the polycationic nature. Quartininized form of chitosan is well known for their better in vitro anticancer ability against Hep3B, HeLa and SW480 cells [35]. However, the natural polymers lack better mechanical strength as well as relatively sudden degradation rate due to its hydrophilic nature which inhibit their use in long term drug delivery process. And also chances for immunogenicity, batch to batch differences, their limited availability, expensive productions and vulnerability to cross-contamination, these all demerits make their clinical application to limited form [36].

On the other hand, the limitation of natural polymers could be minimized in the application by the use of synthetic polymers:mainly biodegradable polymers such as poly caprolcatone (PCL), polyvinyl alcohol (PVA), polylactic acid (PLA), Poligylcolic acid (PLGA). These synthetic polymers can be degraded via enzomolysis or hydrolysis.
Therefore, these materials have great importance in drug delivery because the drug delivery for tissue regeneration process could take time, also the tissue regeneration can occur [37]. The rate of degradation depends on the sustained release of drug so that the degradation rate can be controlled by changing the parameters such as rate of the ratio of amorphous to crystalline segments of polymers and polymer blend compositions [38][39]. Synthetic polymers has many advantages in comparison to natural polymers because of its non-expensive, good mechanical properties, tunable degradation as well as durability. However, they have demerits such as lack of cell specific recognition site due to the hydrophobic and smooth surface.

The production of novel composite fibers in combination of synthetic and natural polymer could reduce the disadvantages. The combination of natural and synthetic polymers would help for the formation of fiber same as extracellular matrix, outstanding mechanical properties and adjustable biodegradability. For example, the PLGA-gelatin was fabricated in blending electrospinning for the drug delivery of fenbufen (FBF) [40]. These blend scaffolds have optimized mechanical properties, degradation rate and bioactivity. However, the drug release profile could be controlled by increasing the PLGA in the blend. It will make the scaffolds more hydrophobic and slower degradation rate. In another paper, blend of PCL-gelatin composite scaffolds was prepared and PCL being a hydrophobic polymer gave rise to tunable hydrophobicity, degradation rate and mechanical properties. Consequently, gelatin provided cellular attachment and adhesion of bone marrow derived from human mesenchymal stem cells (hMSCs). Thus, these type of tunable properties could engender the promising scaffolds for drug delivery application and tissue engineering system [41]. While designing a system for sustained
release of drug, there are many factors contributing for the efficient release of drug from the polymer scaffolds. The factors are degradation and wettability of the polymer scaffolds, type of drug and drug loading type.

For sustained release of the drug, the most important factor is the drug loading type. There are many type of loading including co-axial electrospinning and multilayer electrospinning which shows controlled release of drug for long term. The sustained release of the drug depends on the following factors, in coaxial electrospinning such as thickness of the shell layer, porosity, degradation rate of the shell fiber, hydrophobicity of the scaffolds etc. On the other hand, in multilayered electrospinning the drug release kinetics depends on the scaffolds porosity, thickness of the outer layer and hydrophilicity of the scaffolds etc.

1.5.1.1 Polycaprolactone (PCL)

PCL, known as polycaprolactone, is a hydrophobic polyester polymer widely studied in electrospinning. PCL is having great biomedical application due to its biocompatibility, biodegradation, mechanical property, non-toxicity, low cost, low melting point. Commercially available PCL ranges from 3000 to 85000g/mol molecular weight. PCL is a hydrophobic molecule. Hence, it dissolves in solvents like chloroform, acetone, acetic acid, dichloromethane, toluene, methanol, benzene and tetrachloride [42]. PCL properties like biodegradability, cytotoxicity and degradation rate has been studied elaborately for short and long duration in implantations [7], [8]. Degradation of PCL is non-enzymatic by hydrolysis. PCL fibers are well studied as drug carrier in drug delivery.
1.5.1.2 Polyvinyl alcohol (PVA)

PVA known as polyvinyl alcohol is a semicrystalline hydrophilic polymer which is easily soluble in water. The solubility in water makes the PVA a great application in drug delivery. PV is a biocompatible, biodegradable and easily electrospinnable polymer. PVA has been used for sacrificing template for the preparation of non-electrospinnable polymers. However, individual PVA cannot be used for drug delivery due to its water solubility. As it will lead to burst release of drug. PVA was fused with chitson to improve its biocompatibility and cell attachment [5][6]. Gelatin electrospun with PVA as template for better fiber of gelatin[43]. PVA has been used for sacrificing template for the preparation of non-electrospinnable polymers[43]. However, PVA have poor mechanical properties. Therefore, many scientists have tried to study the composite material which could enhance the mechanical properties of the PVA[43]. PVA embodiment with PCL polymer has gained much attention recently because its mechanical character could be enhanced by the addition of PCL. Therefore, PCL/PVA as multilayers scaffolds for sustained and controlled release of drug was studied below.

1.5.1.3 PCL/PVA:

The multilayered structure has gained much attention due to its versatility and controlled release of drugs. The drug was studied as middle layer and outer layer would controlled release of antibiotics. For instance, Yuan-Yuan Liu [44] prepared a novel scaffold by integrating 3D bioprinting platform and electrospinning for studying the multiple drug delivery. Here, PVA blended with gentamicin sulfate and co-axial PVA-DFO/PCL was fused like layer by layer to form a 3D scaffold for osteointegration and sustained drug release. Burst release was noted for gentamicin sulfate, but sustained and controlled
release for DFO due to the presence of vertical gradient of sodium alginate/gelatin in the scaffold makes the DFO release as gradient- mode. Therefore, combination of 3D bioprinting and electrospinning can be used for preparing functional gradient scaffolds. In another study, release of the model drug tetracycline hydrochloride (TC-HCL) and phenytoin sodium from the PVA-PCL-PVA multilayered electrospun nanofibers was reported[45]. The hydrophilic and hydrophobic polymer was prepared layer by layer by incorporating multiple drugs such as PHT-Na to OVA and TC-HCL to PCL, respectively. The 87% of TC-HCL was released from single fiber and only 47% was released from multilayer scaffolds. The release kinetics mechanism was fickian diffusion as well as release profile corresponded to korsmeyer-peppas equation. These materials had great application in wound dressing mats. The multilayered electrospun fiber scaffolds have great importance in drug delivery.

1.6 Type of drugs

1.6.1.1 Antibacterial and antibacterial agents

Antibiotics and antibacterial agents were incorporated for the enhancement of scaffold properties. Iganatova.et al. has studied the use of diverse antibiotics in the electrospun scaffolds and their application of wound dressing [46]. The antibiotics are tetracycline hydrochloride, ciprofloxacin, moxifloxacin, levofloxacin and antibacterial agents (for example, 8-hydroquioline derivatives, bezalkonium chloride, itraconazole, fusidic acid or silver nanoparticles). Gentamicin sulphate loaded PLGA and gelatin was also studied for the continuous release of drug [47]. The results showed that PLGA/gelatin 70:30 nanofibers scaffolds exhibited a systematic release in the drug during first 15h rather than burst release effect. This indicates that this is a promising scaffold for wound healing
applications. On the other hand, the drug release profile was studied for polyethylene
covinyl acetate and PLA blend scaffold in which TC-HCl was the model drug[48]. The
release of the drug delivery depends on the type of fiber and percentage of drug content.
The result stated that the 5% drug loaded 50/50 blend solutions had shown drug release
for 5 days without burst release. In addition, 25wt% has indicated rapid release than the
5wt% due to the more surface-partition effect of the drug in the former case.

1.6.1.2 Anticancer:

Not only antibiotics but also many other types of drugs, such as anticancer drugs, are
applied to the scaffolds of electrospun mats for chemotherapy. Diverse anticancer drugs,
such as docorubucin (Dox), paclitaxel (PTX), dichloroacetate and platinum complexes
are incorporated into the electrospun fibers for localized postoperative chemotherapy
session. For instance, Xu et al. fabricated PEG-PLLA loaded electrospun fibers via
emulsion water-in-oil emulsion method in which aqueous phase was hydrophilic drug
and oily phase was chloroform solution of PEG-PLLA. The results showed that, PLLA
was completely immersed in the electrospun fibers[49]. In the same way they
successfully incorporated hydrophobic Pacitaxel (PTX) and DOX, simultaneously added
to the nanofibers scaffolds via emulsion electrospinning method, and then multiple drug
delivery was studied [50]. In contrast, Xe et al prepared electrospun scaffold of
PLA/PLGA (30/70) blend fiber added with cisplatin, and the results showed a 90%
encapsulation efficiency; the sustained release of drug was noted for 75days to treat in
vitro of glioma [51].
1.6.1.3 Protein, DNA, RNA and other growth factors

Overtime, electrospinning has improved; thereby propagating many new innovative ideas for biomedical applications. Blend electrospinning and co-axial electrospinning has been developed with the addition of protein, DNA, RNA and growth factors combined with the electrospun fiber mats for biomedical applications. The main challenges faced in this type of design is the loss in the bioactivity of the drug incorporated. Therefore, it is mandatory to optimize the material and electrospinning parameters for efficient results. Hence, the process of blend electrospinning and co-axial electrospinning has acquired more interest in this specific type of drug addition. Co-axial electrospinning is more efficient for protecting the bioactivity of the drug than blend. Chew et al, encapsulated the human nerve growth factor with the BSA as a carrier into the polymer such as PCL and poly(ethyl ethylene phosphate)[52]. The results showed that, there was a partial bioactive retention of the hNGF when the PC12 cell line was introduced to the scaffolds. There was a consistent release of hNGF around 3 month without burst release. The same group studied the release of small interfering RNA (snRNA) and transfection reagent (TKO) on electrospun fibers of copolymer caprolactone and ethyl ethylene phosphate (PCLEEP) [53]. The results showed a sustained release of siRNA around 28 days. The copolymerization of ethyl ethylene phosphate with PCL has induced the SiRNA delivery rate as well as gene knock down efficiency than PCL alone. In co-axial electrospinning the bioactive components are incorporated inside the core and protected by shell polymer. Hence, bioactivity can be protected to the electrospinning environment and biological environment. Saraf and co-workers have studied the addition of plasmid DNA (pDNA) in to the core and shell polymers with non-viral gene carrier poly(ethyleneamine)-hyalouric
acid (PEI-HA)[54]. The gene release was notified around 60 days by diverging the parameters such as concentration of pDNA and molecular weight of the core for controlling the transfer efficiency of the pDNA. The Bioactivity of the drug could be controlled by the new design suggested by Mickova et al [55]. He proposed the addition of liposomes to the core which can hold the bioactive ingredients and protect its activity for efficient action due to the shielding of lipid sphere from the electrospinning process.

1.7 Mechanisms of drug release:

The drug release mechanism from the scaffolds are completed in three process such as desorption from the surface, diffusion through the fibers and fiber degradation[56]. These three processes could occur together which impacts the release kinetics throughout the entire process. The Figure (3) illustrates the schematic representation of drug release behavior from different types of dug loading. When the fiber is immersed in the aqueous media desorption mechanism happened to the drug on the surface as well as drug present inside the nanopores of the nanofibers [57] . In these three mechanisms, desorption is considered for the drug on the surface of the polymer, therefore burst release is noted. This burst release is due to the direct interaction of the medium with the polymer surface. Hence, burst release of the drug is not useful so surface modification is obtained, i.e. The main physical modification for the controlled and sustained release of the drug to the environment.

The second type of kinetics is diffusion mechanism, in which the concentration gradient makes the release of drug to the medium. Herein, diffusion process reduces the effect of initial burst release and exhibit controlled and sustained release of drug. The co-axial and emulsion electrospinning model can showcase this type of release kinetics. Finally, the
third type of release mechanism is degradation of the outer surface. For instance, low degradable polymer used as the shell showed sustained release of drug due to low degradation rate. Therefore, the mechanism of drug release kinetics was optimized using the polymer incorporated and the type of electrospinning process. PCL is a low biodegradable polymer however; PVA is a highly biodegradable polymer. Therefore, combination of these two polymers could provide a better drug release profile.
Figure 3: Drug loading and release (desorption and diffusion) from polymeric micro/nanofibers fabricated by (a) surface modification, (b) blending, (c) coaxial and (d) emulsion electrospinning. The green color stands for polymer, blue for drugs and maroon for surfactant. The red arrows represent the direction of the drug release [58].
1.8 Tissue Engineering

Electrospinning techniques have gained much consideration in tissue engineering. The electrospun fibers structure possesses many characters suitable for tissue engineering such as mechanical properties, high surface to volume ratio and adjustable porosity. Tissue engineering is an advance field of science which merges with applied engineering and bioscience for constructing biomaterials that recover, sustain or improve the biological activities of injured tissues [9]. For efficient tissue engineering process, three parameters are considered such as, seeding and attachments of cells, biomaterial scaffolds and addition of cell signaling factors. In this, biomaterial scaffolds is a major parameter where they should mimic natural extra cellular matrix, sufficient mechanical properties, biocompatibility, hydrophilic surface, biodegradability, high surface area and high interpore connectivity. These criterions which contribute to cell proliferation, differentiation and migration. In this, electrospun fibers can be prepared in cost-effective and efficient manner to produce suitable candidate scaffold for tissue engineering. Nowadays, several electrospun fiber mats are prepared and studied for tissue engineering with or without addition of biological agents or growth factors for wound healing, bond construction and nerve tissue regeneration.

1.8.1 Skin tissue engineering/ wound healing

In recent years, wound healing or skin tissue engineering has been researched very often. Generally, the wound healing process takes place in four different steps: hemostasis, inflammation, proliferation and remodeling [10]. The wound healing is stabilized by different cells, growth factors and cytokines. In the first process, the host cells and
bacteria are removed by the macrophages in the inflation step. These macrophages also secrete some factors eligible for the angiogenesis, fibroplasisa and extracellular matrix production [11]. The proliferation, migration and remodeling of endothelial cells is an important factor that contributes to angiogenesis [40]. Finally, fibroblast proliferation leads to the rebuilding of the function and structure in the injured site[13]. Therefore, efficient wound dressing material is mandatory for proper treatment of the wound. Wound healing scaffold should have good biocompatibility, mechanical properties and capability to prevent the fluid evaporation from the injured site. Furthermore, it should provide the site for cell epithelization and inhibit the infections[59]. Hence, the ability of cell attachment to the electrospun fiber scaffolds plays an important role in the efficiency of engineered wound dressing scaffolds. Material and manufacturing process are important for the preparation of an ideal wound dressings mats. Electrospinning is an ideal manufacturing process for wound dressing mats due to the above advantages such as it has biocompatibility, biodegradability, hydrophilic surface, porosity and so on. Moreover, as nanofibers scaffolds grant a better clearing of exudates from the injures site, manages the loss of water and also oxygen diffusion in and out of the wound site [60]. There are natural (like collagen, gelatin, chitosan) and synthetic biodegradable polymers (PCL,PLGA,PGA,PLA, PVA etc) that are molded together to form scaffolds. Electrospun scaffold are prepared in combinations of different natural and synthetic biodegradable polymers loaded with antibacterial and wound healing factors. The polymers are co-electrospinned, blended, co-axial and multilayers electrospinning. Syed Mahdi Saeed [61], has studied prepared a multilayered fiber mat loaded with curcumin as an antibacterial active component from the novel PCL-PVA-PCL multilayered
electrospun fibers. The results show that, multilayered PCL-PVA curcumin-PCL has illustrated better exudate absorbance than pristine dressing in the incision. In the same vein, it summarize that, 16% loaded curcumin display antibacterial activity without killing the cell viability. Antibacterial properties can be built up in the scaffold with the addition of antibacterial agents or antibiotics. Silver is a well-known antibacterial agent because it can damage the DNA replication of bacteria [18]. Fatemeh Khodkar [62] has successfully prepared PVA/PCL core/shell loaded with silver nanoparticles in core for wound dressing applications. Fiber loaded with silver shown lower porosity as well a water vapor transmission rate (WVTR) and also greater contact angle. These scaffolds are suitable for long term antibacterial activity (Escherichia coli and Staphylococcus aureus) because of the sustained and controlled release of the silver nanoparticles in core/shell structure. On the other hand, PCL/PVA was co-electrospunned by loading silver sulfadiazine (SSD) as drug for wound dressing mats [18]. PCL and PVA loaded with SSD was prepared successfully. The effect of different weight % of SSD on mechanical and cell toxicity and antibacterial properties was studied. The better SSD concentration was identified with antibacterial ability and cellular attachment as well as proliferation was notified. Where, addition f SSD increases the fiber diameter as well as hydrophilic properties but reduces the mechanical properties of the scaffold. Fibronectin coating can improve the biocompatibility of the scaffolds loaded with SSD. Therefore, 5% SSD loaded co-electrospun PVA-PCL show better antibacterial and reasonable cell proliferation and differentiations. Recently, L. Du has fabricated PVA merged with monodisperse AgNPs and PCL loaded with Ascorbyl palmitate (AP) by duel spinneret electrospinning [63]. The NIH-3T3 fibroblast cells are seeded on the scaffold mats and
showed that, AP inhibits the toxic effects of AgnPs on cell proliferation. It should also be noted that, antibacterial tests validate the inhibition towards gram negative and gram positive Escherichia coli (E. coli) and Staphylococcus aureus (S. aureus) respectively. Wound healing test and histological observation conclude that, this material provide a promising for future biomedical applications.

[64] Porosity and surface wettability is an important parameter which determines the healing process. Xin Liu has electrospun PVA, PCL, PAN and PVdF-HFP incorporating wool protein and Ag for wound dressing mats. Thus, it can be concluded that, other than nanofiber diameter and antibacterial property the porosity and hydrophilicity is an important factor that enhances the wound healing process. The Hydrophilic membrane showed efficient remedy for wounds in comparison to the hydrophobic membrane. Porosity for oxygen diffusion also leads to better wound healing process. However, wound healing process for diabetic ulcers are time consuming due to the lack of efficient blood supply because of higher amount of sugar in the blood. These processes leads to long inflammatory stage, defected angiogenesis and blocked fibroblast proliferation. Adeleh Gholipour-Kanani has prepared novel electrospun blended fibers of Chitosan-poly (vinyl alcohol) (Cs: PVA) (2:3) and poly (caprolactone)-chitosan-poly (vinyl alcohol) (PCL: Cs: PVA) (2:1:1.5) for wound healing in diabetic patients. The above fabricated scaffolds wound healing prate was studied by applying to diabetic dorsum skin wounds and diabetic foot wounds on a rat model (n-16). The scaffolds had far effective healing process than control ones. More granulation tissues are presence in the scaffolds treated wounds than the control ones and within 20 days the scaffolds provided better
wound repair than controls. Hence, PVA:CS:PCL is a considerable material for diabetic wound healing.

1.8.2 Bone tissue engineering applications:

Bone is the strong rigid organ which plays an essential role in our body. It protects our vital interior organs, movement, manufacturing of white blood cells and red blood cells, and also storage of minerals[65]. In bone extra cellular matrix mainly consist or organic and inorganic components such as collagen and hydroxyapatite (HAp). Incorporation of these components make suitable scaffolds for bone tissue engineering applications. The electrospun scaffolds architecture play an important parameter for successful bone regeneration. They are microstructure of the scaffolds, its porosity and surface properties[66]. The electrospun fiber should provide better mechanical properties to support the structure and arrange a space for the osteochondral adhesion, proliferation and differentiation. Hence, the development of an ideal scaffold for tissue regeneration could be achieved by using a porous ceramic material, lamellar material and a fiber matric material for better biological and physical properties. Subramanian Uma Maheshwari has developed a scaffold comprise of polymer – ceramic combination of PCL/PVA bilayer scaffold blended with HAp nanoparticles[67]. (PVA-PCL)-HAp has improved its porosity around 64% as well as hydrophilicity around 141%. Also MTT assay studies with MG-63 osteoblast cells had better cell adhesion and proliferation which show a promising application for tissue regeneration. However, incorporation of growth factors (GF) or drug to the scaffold is also important for enhancing the regrowth of broken bones. There are many GF such as bone morphogenetic protein-2 (BMP-2) and VEGF added to the electrospun scaffolds for the long lasting sustained release of GF to mimic
the natural healing process. For instance, co-axial electrospun of collagen-PCL incorporated with BMP-2 and dexamethasone (DEX) has shown better controlled release of GF, thereby encouraging the osteogenic expression of human mesenchymal stromal cells hMSCs [68]. In this design the shell layer was loaded with DEX and the core was incorporated with BMP-2. The dual drug release was exhibited, in which DEX shown fast release. However, BMP-2 demonstrated sustained release over 22 days. This scaffold provide healing process as well as osteogeneration efficiency.

On the other hand, incorporation of stem cells into the biomaterials is also a novel approach for tissue regeneration of the cells. For instance, Abbas Shafie has studied in vitro and vivo of the cartilage tissue regeneration from rabbit bone marrow mesenchymal stem cells (BM-MSC) seeded on electrospun scaffold of PVA/PCL nanofibers[69]. In vitro, the MTT assay shown that, the scaffolds backing the chondrogenic differentiation of MSC. In vivo, the scaffold with and without MSC loaded were implanted on a rabbit full-thickness cartilage defects. To study the cartilage regeneration, histological and semi-quantitative grading was executed. The results shown that, scaffold seeded with MSC has enhanced the healing process as compared to non-seeded scaffolds. These results show that, PVA/PCL scaffold seeded with MSC is suitable for graft for articular cartilage repair.

1.8.3 Skeletal muscle regeneration:

Skeletal muscle made up of around 40% of human body. Skeletal muscle is made of various fibers with diameters ranging from 10 to 80mm[70]. These fibers are unidirectional and produce enormous amount of force during contraction [71]. If a muscle cell gets injured or wounded, it will not be possible to contract, satellite cells are
switched on its activity for muscle cells regeneration. However, this healing process would create a scar in the tissue and block the muscle function [71]. There are many efforts took place to study the initial steps for muscle regeneration such as autologous muscle transplant, satellite cells, exogenous myogenic cells and myoblasts but these methods met finite success[72]. Therefore, long term denervation and severe injuries can leads to the loss of skeletal muscle activities.

Muscle tissue engineering materials desire better contracting ability and mechanical properties [73]. Muscle cell adhesion and proliferation has been studied using both mechanical properties and electric stimulus in the cell culture. Mckeon-fischer K D has prepared co-axial electrospun fibers with the core as PCL and Multiwaled carbon nanotubes (MWCNT) and blend of (83/17, 60/40, 50/50, and 40/60) poly(acrylic acid/poly(vinly alcohol) (PAA/PVA) as the modified outer shell layer [74]. All the four components were electrically conductive, although, the scaffold didn’t show a actuated when electric field is applied. The best result was shown at 20V. The MTA assay by Soleus and vastus lateralis (VL) muscles extracted from rats, result shown that, 0, 0.14% and 0.7% of concentration of MWCNT in the scaffold has not toxic for the cells over 4 weeks period. According to different percentages of blend solutions PAA/PVA in the outerlayer 40/60 has illustrated higher number cells than other scaffolds. Scaffold has tensile properties that are higher than the skeletal muscle’s. More modification of these scaffolds for contraction rather than bending can lead to promising scaffolds for artifical muscle applications.
1.8.4 Nerve tissue engineering:

Electro-conducting polymers such as polypyrrole (PPy), polyaniline (PANI), polythiophene (PT), poly(3,4-ethylenedioxythiophene) (PEDOT)) show attractive electrical and optical phenomena. Thus, they have been researched in the past few decades for various applications such as microelectronics, actuators and polymer batteries[75]. The electro-conducting polymers which hold the specialization of biocompatibility and good conductibility can be applied as biosensors and tissue engineering scaffolds [76]. The electrospun electro-conducting polymer have great deal for electrically stimulate neurons and nerve tissue engineering as well as neural prostheses application for therapeutical function [76][77]. Schmidt et al has firstly studied the PC12 cells through polypyrrole (PPy) electroconducting polymer, recognize the growth of PC12 cells on the thin film of PPy these enhance the neurite outgrowth from the cells, these results suggest the greatest application of these type of scaffolds for nerve tissue regeneration [78]. Many studies have been suggested improvement of the electro conducting polymer for nerve tissue regeneration application by adding cell adhesive[79], neurotrophins[80] and topographical features[81]. Jae Y Lee has prepared electrospun nanofibers coated with conductive polymer PPy for nerve tissue engineering applications[82]. The PPy-PLGA improved the growth of rat pheochromocytoma 12 PC12 cells and hippocampal neurons than that of non-coated PLGA as control. This suggest that PPy-PLGA would use for the nerve tissue engineering application. Simultaneously, electrical stimulus studies on the scaffold suggest that, at stimulus of 10mV/cm has improved the neurties to 40-50% longer as well as increment of 40-90% more neurons formation compared to non-stimulus at same scaffolds. Moreover, aligned scaffolds show more neurites elongation
and formation than that of randomly oriented PPy-PLGA fiber scaffolds. These results suggest that, good response for electric stimulus and biocompatible polymers prepared by electrospinning have great advantages for biomedical applications such as nerve tissue engineering.

1.9 Sputtering technology for biomedical applications:

Plasma technology is a polymer that has improved the surface properties of the polymers without changing their bulk characters. The plasma treated polymer had found great application in diverse fields such as automobiles, microelectronics, chemical and biomedical industries[83]. The polymer surface properties such as hydrophobicity, roughness, chemical structure, conductivity etc. can be molded for various applications. The plasma treatment could affect the polymer surfaces by micro-etching, organic contamination, cross-linking, surface chemistry modification and surface coating with specific target material[84]. The biomaterials should possess a good mechanical and surface characters eligible for biological environment. For instance, for cell adhesion, the polymer surface should have low surface free energy, surface roughness and hydrophilicity matters. The plasma treatment via magnetron sputtering technology has been reimagined by coating the surface of polymer to form biomaterial suitable for biomedical applications such as antibacterial activities, biocompatibility and tissue engineering.

There are thermal and non-thermal deposition process in plasma sputtering technology. However, non-thermal deposition procedure is highly recommended for polymers because it will not damage the bulk properties of the polymer. Magnetron sputtering is the technique used for coating the polymer surface. Magnetron sputtering is a technology
developed during 1970’s it is a high-speed and low temperature technique for preparing uniform and strong adhesion film on the surface of polymer, ceramics and composite materials [85]. Sputtering is simply a call as momentum transfer between the particles [86]. Wherein, when an positive ion from sputtering gas (plasma) with high speed strike on the surface of target materials and surface atoms is ejected from the target and deposited on the substrate[87][88] when the environment is electric and magnetic field. Argon, nitrogen and oxygen are the sputtering gas. However, argon gas is commonly used because they don’t damage the target due to its nobility. The ejected atoms are accumulated on the substrate via adjusting the distance between the target and substrate. This process leads to the formation of thin film on the surface of the polymer. The full process is very fast and requires only low temperature, and high film forming rate as well as strong film adhesion[89]. For instance, the composite microfibers of Poly(methylmethacrylate/ organically modified montmorillonite (O-MMT) was manufactured by electrospinning incorporation with emulsion polymerization[90]. Here the prepared composite microfibers of PMMA—O-MMT was magnetron sputter coated with Titanium dioxide (TiO2). The results showed that, increased deposited anatase-TiO2 and rutile-TiO2 has shown better wettability of the surface without damaging the PMMA—O-MMT compound. These composite fibers have UV absorption 254nm. Therefore, it has the tendency for photocatalytic degradation of model compound methylene blue. Thus, these materials provide a promising application in dye waste water treatment.

The polymer microsphere[91] , thin film[92] and fibers[93] are sputter coated with Ag[93][94], Cu[95], Ti[96], TiO2 [97], gold[98], Hydroxyapatite (HAP), tricalcium
phosphate (TCP) [99], amorphous calcium pyrophosphate (CPP) [99] and dicalcium phosphate dihydrate (DCPD) [99] for various polymers in microsphere, thin film and electrospun fibers for different biomedical applications such as antibacterial and tissue engineering applications are discussed below in details.

1.9.1 Antibacterial coatings:

Attachment of bacteria to the surface of a polymer can lead to the formation of biofilm. Therefore, biofilm resistant polymers for medical field are an important factor. Biofilm resistant could be an assistance to the polymer by the addition of antibacterial agents on the surface of polymer to prevent the bacterial adhesion. The materials such as medical textile, wound dressing, prosthesis and implant materials should display antibacterial activity for efficient biological activity. Antibacterial properties are an important parameter to take into account for wound dressing. The antibacterial activities are promoted by the addition of some antibacterial components to the fabrics. There are many components such as inorganic and organic (drugs) as well as metal. The inorganic agents include titanium dioxide, carbon nanotubes, silver, zinc, zinc oxide, copper, gallium and gold nanoparticles [100]. Organic includes some antibiotics [101].

Among the inorganic antibacterial components silver nanoparticles was well studied [102]. The silver nanoparticles was added in the electrospun fibers via Ag ions through wetting process[16][17], silver sulfadiazide [18], etc. The wetting process for the preparation of Ag to the matrix has many disadvantages such as uneven distribution of nanoparticles, using reducing agents that are toxic, and controlling the size of nanoparticles is a difficult task depending on the strong and weak reducing agents used[19]. However, the most efficient way of introducing nanoparticles to the surface of
polymer or fabrics could be done by using plasma technology. Plasma technology provide uniform deposition, less use of resources as well as simple process for the coating of antibacterial material such as Ag, Si, Cu etc, to the surface of the polymer than wetting process. Sputtering known as physical vapor deposition has been effectively used in coating diverse thin film in electronics applications. Therefore, sputter coating of metals to enhancing the antibacterial properties are contributed by addition of many target materials such as Ag, Ag/Si, Cu, Ti etc.. Therefore, more studies are required to compare the antibacterial properties of various materials incorporated with bioresorbable polymers.

1.9.1.1 Silver (Ag):

Silver is a transition metal in the periodic table. Silver related compounds or nanoparticles have biocidal effect on around 16 specious bacterial because of its toxic effect on microorganisms [14][15]. Thus, silver is coated on medical devices for antibacterial properties [103]. At low concentration silver nanoparticles show good antibacterial efficiency [104]. Moreover, the in-toxicity effect of AgNP to human monocytes cell lines show the application of Ag to the fabrication of medical devices.

The mechanism of antibacterial activity of Ag on microorganism are not studied well yet. It has been shown that in E.Coli the AgNP treated bacteria has shown some pits on the cell wall and accumulation of Ag in the cellular membrane. This type of membrane has shown increase in the permeability. Bacterial DNA drops its replication ability as well as cellular protein and becomes denatured by binding Ag ions or nanoparticles to the functional group of the protein[105]. This results in the cellular death of bacteria. On the other hand, some reported that Ag nanoparticles will denatures cellular protein suitable
for the cellular nutrient transport and damage the cell membrane or cell wall, enhanced cell permeability and followed by cell death [15]. It is also noted that, the antibacterial efficiency of Ag depends on its shape. Ag Nps with \{111\} plan lattice basal plane show strongest antibacterial action than spherical and rod-shaped nanoparticles and silver ions [106].

Silver has good antibacterial properties. Silver treatment is well known for wound dressing materials. The silver is incorporated as silver nanoparticles by the introduction through AgNO3 by using reducing agent. However, this type of silver incorporation leads to burst release of silver from the material with very high concentration of Ag in the wound. These is followed by the sudden reduction of silver because of both bacterial consumption as well as reaction with other compounds present in the wound beds such as phosphates, chlorine and proteins. Therefore, silver release in the wound should be in a controlled manner. In addition, silver nitrate present is an hypotonic and hence it can effect strong electrolyte imbalance which could damage the wound site, produce gross systematic imbalance which could kill the patients with heavy burns who require large doses of silver. But silver sulfadiazide was developed in accordance to minimize the side effect of using silver nitrate. However, the removal of silver sulfadiazide cream from the wound surface is by scrapping, it could proclaim the patients painful dressing procedure. Moreover, sulfadiazide does not show any hypotonic effect. Therefore, it is mandatory to have a better procedure for delivering silver. The efficient way of introducing silver is via prolonged period, act against many range of bacteria, require only few changing wound dressings, and never interfere with the wound healing process. Thence, sputtering is a new field of surface coating of wound dressing materials for prolonged release of silver
with efficient antibacterial properties. The optimization of sputtering process is an important criterion for better antibacterial properties.

It is also noted that, silver is the best candidate for wound curing application because it will reduce the inflammation [107] and impedes the contraction and improve cell epithelialization [108]. The silver nanoparticles are toxic to effect on cell viability these leads to decrease in biocompatibility of scaffolds [109]. However, the amount of Ag to the scaffolds can be optimized with the antibacterial effects and cellular toxicity of Ag.

Antibacterial coating on medical textiles is an important criterion to avoid infections during surgical process[110]. The silver is coated on textiles via different techniques. But it has also reduced the durability of the coatings, which were very poor, hence limiting their usage. Therefore, strong adhesion of silver on fabrics is required via sputtering coating technique [110]. Cotton fabric with antibacterial properties could have various applications. Silver is the most commonly used material to enhance the antibacterial properties in cellulosic fibers [110]. Silver incorporation could be made wet or dry process. The wet process is change the bulk properties of textiles and also has negative impact to the environments. However, dry process such as sputter coating is an ecofriendly process which only changes the surface of the matrix. Therefore, Ag was cooperated into the cotton matrix in various thickness and studied the antibacterial, release of the Ag in water etc. The results suggested that, Ag has shown antibacterial activity against Staphylococcus aureus, Escherichia coli and Candida albicans [110]. And also sputter coating has improved the water contact angle of the cotton fabrics. Thus, these ideas sputtering could be added to nanofibers for antibacterial activity. Simultaneously, Ying-Hung Chen has coated sputter coated the PET fabrics in high
power impulse magnetron sputtering which provide highly concentrated plasma so these will support strong adhered films[111]. These results shown that, the sputter coated more than 1 min films has shown great antibacterial performance via bacteriostatic (>2.0) and bactericidal (>0) effects, based on JIS standards. Furthermore, the coated fabrics have shown capability to hold the antibacterial properties over 20 cycles of washing, indicating long-term durability for the fabrics.

The wound dressing mats of polymer sheet and electrospun fiber scaffolds was sputter coated with Ag and studied its antibacterial properties. Xiuju Liu has studied the influence of magnetron sputter coating of nano-silver on polyetheretherketone (PEEK) and studied its cytotoxicity and antibacterial properties[112]. PEEKs were sputter coated with Ag 3nm, 6nm, 9nm and 12nm. The antibacterial properties and bacterial adhesion to the surface was studied. The homogeneous Nano silver was coated on the surface and it shown that, water contact angle was increased as well as there was no cytotoxicity for the CCK-8. And also the coating provides good adhesion of bacterial towards PEEK and also improved antibacterial activities towards Streptococcus mutans and Straphylococcus aureus.

The combination of electrospinning and sputtering technology can make many novel composite fibers with diverse applications in biomedical such as wound dressing mats with good biocompatibility and antibacterial properties. The electrospun microfibers was coated with Ag by DC magnetron sputtering [94]. The electrospun scaffolds of poly(glycerol sebacate)/poly(3-caprolactone) (PGS/PCL) was coated with Ag and its antibacterial properties and silver release behavior was studied. PGS/PCL shown good mechanical and thermal behavior due to increase in fiber diameter and decrease in fiber
pore size when sputter coated with Ag. The fiber scaffolds has shown gradual release of Ag which contribute for antibacterial activity. Therefore, this material could possess good application in wound dressing and bandages. Moreover, implant prosthesis also requires antibacterial properties to avoid infection after surgery. Incorporation of Ag with SiO₂ was used in implantic prosthesis after hernia repair can leads to infections in the abdominal area, therefore prevention is an important goal. Giuliana Muzio has studied the effect of polypropylene prostheses coated with silver-silica composite (Ag/SiO₂) layer by sputter coating technique for improving the substrate antibacterial and biocompatibility of the prosthesis [113]. The prepare meshes hernia prostheses (CMC) consist of two layers microporous light mesh and thin transparent film of polypropylene. They have sputter coated the Ag/SiO₂ composite on CMC meshes and microporous mesh layer alone. The sputtering process was optimized via addition testing biocompatibility and antibacterial properties. And it is noted that, sputter coated with CMC improve antibacterial property but reduced biocompatibility. However, sputter coated meshes alone show good antibacterial and biocompatibility. And also, fiber meshes coated with Ag/SiO₂ enhance the growth of seeded fibroblast without creating apoptosis or necrosis of the fibroblast in addition meshes exhibited good antibacterial property as well. 

1.9.1.2 Copper (Cu):

Other than Ag the electrospun scaffolds was sputter coated with Cu for antibacterial property [114][115]. Cu is cheaper than Ag therefore Cu coating can provide economical wound dressing mats. PLA scaffold was DC magnetron sputter coated with Copper (Cu) [95]. The PLLA scaffold has increased the hydrophobicity in proportion to the plasma treatment time. The antibacterial testing concludes that, modified composite scaffold
have bacteriostatic effect in which 30 and 50% of bacterial has reduced. And also it is found that copper have strong antibacterial effect than copper oxide. Therefore, this type of composite material can be used for economically cheap wound dressing mats for having antibacterial effect.

### 1.9.2 Tissue engineering:

Plasma technology has also emerged recently on tissue engineering application such as vascular grafting, stem cell therapy, artificial muscle sputter coated with conductive gold. Therefore, the good biocompatible polymer had provided a platform for cell adhesion, proliferation and differentiation. Therefore, if stem cells are added to the polymer scaffold which can provide better tissue regeneration environment. Stem cell therapy is a new platform for substituting many complicated surgical procedures. Stems cells loaded materials has gained much attention recently [91]. Here in, PCL microsphere incorporated with sputter coated Au has used as a platform for the differentiation of cardiomyogenic cells from human embryonic stem cells. Presently, here they demonstrated the PCL microsphere sputter coated with gold particle for 5min and without coating [91]. Then they added human embryonic stem cells (hESCs) to the microsphere. And it noted that, AuMS shown higher cardiac differentiation in because Au play as mediator for expressing genes on day 4 and day 14 which are suitable for the cardiomyogenic differentiation than MS. These papers also suggest AuMS is non-cytotoxic material.

Moreover, The PLLA and PEG fibers was electrospun and sputter coated with calcium phosphate for bone tissue engineering applications. Here, they just combined
electrospinning and sputtering technique are feasible for fabricating biopolymer scaffolds for biomedical applications[116].

There are many novel composite material has emerged due to the fusing of two precious technique such as electrospinning and sputtering technique[117]. A novel material for vascular tissue engineering was studied. These composite materials was prepared by electrospinning and sputtering technique. In this, beresosable polymer PCL and PHBV was incorporated in 1:2 (v/v) and sputter coated with Ti. Firstly, they optimized the sputtering process which would not damage the macro structure of the scaffolds. The biocompatibility of the prepared composite mats was studied with hybridoma of the endothelial cells of human umlical vein and human lungs carcinoma (EA.hy.926 cell line). The results from cell adhesion, viability and secretion of IL8 interleukin-8 (IL8), interleukin-6 (IL6) ang vasculat endothelial growth factors (VEGF) demonstrate that cell adhesion has improved for ti coated scaffolds as well as it determine that novel scaffolds have better proangiogenic activity.

And also a novel approach was incorporated to make a fiber scaffold suitable for artificial muscle or smart devices for human body was synthesized by using electrospinning and sputtering technology[98]. A core-shell structure was made via first electrospinning the PMMA in optimized form to get a uniform fiber and later PMMA was coated with gold for inducing the conductivity and mechanical properties of the scaffolds. Later on, the polyaniline (PANI) was coated on the scaffolds via in situ electrochemical polymerization starting with aniline and using sulfuric acid as oxidizing agent Figure (4). PANI coated metalized fiber scaffolds in similar to core-shell structure has shown fascinating electrochromic properties where color changes occur when applied
voltage was simultaneously switched from 0 to 1V and vice versa. In vitro biocompatibility test reveal good cell adhesion tested on human amniotic fluid stem cells show a better for eukaryotic cells. Moreover, PANI coated exhibit high cell adhesion property. Therefore, this type of web could be used for preparing smart artificial muscle devices via simple and versatile preparations technique such as electrospinning and sputtering.
Figure 4: Schematic representation of electrospinning and sputtering of electrospun for muscle tissue engineering applications [98].
1.10 Materials

1.10.1 Poly(caprolactone) (PCL)

Poly(caprolactone) PCL was first invented in the 1930s and it became commercially accessible due to the property of a synthetic polymer decomposed by microorganisms[118]. PCL has a low melting point (59-60°C), a hydrophobic and semi-crystalline polymer which has good solubility in organic solvents such as benzene; toluene and cyclohexane as well as it can be easily blended with other polymers. This property makes PCL great attention in biomedical applications and also PCL is an FDA-approved for biomedical uses[119]. PCL was synthesized by ring open polymerization of monomer ε-caprolactone incorporation with low molecular weight alcohols to monitor the molecular weight of the polymer Figure (5) [118]. PCL decompose by hydrolysis, where the byproduct is suitable for natural metabolic pathways as well as it will not change the surrounding pH [119].

![Chemical equation for the synthesis of PCL](image)

Figure 5: Chemical equation for the synthesis of PCL
1.10.2 Poly vinyl alcohol

Poly (vinyl alcohol) is a hydrophilic polymer synthesized industrially by hydrolysis of polyvinyl acetate as shown in the equation 1 below:

\[
[\text{CH}_2\text{CH(OAc)}]_n + \text{C}_2\text{H}_5\text{OH} \rightarrow [\text{CH}_2\text{CH(OH)}]_n + \text{C}_2\text{H}_5\text{OAc}
\]

There are different grades of PVA available commercially. They are fully hydrolyzed and the partially hydrolyzed PVA build upon the amount of acetate group left the backbone of the polymer structure [120]. PVA is a hydrophilic polymer with good biocompatibility and toxicity. Also, its good chemical stability, physical (melting point 200°C) and mechanical properties has led to many applications such as cosmetics, medical, pharmaceutical, food and packing industries. Ultrafine PVA fibers are produced by electrospinning process which had obtained potential application in biomedical engineering. In drug delivery, PVA can hold many hydrophilic antibiotics and drugs. Also it is noted that incorporation of PVA with a hydrophobic polymer such as PCL can be used for sustained release of drugs in a suitable manner [121].

1.10.3 Gentamicin sulphate:

Gentamicin sulphate Figure (6) is an antibiotic invented in 1963 from micromonospora purpurea [122]. It belongs to the aminoglycosides family. The presence of hydroxyl group makes it easily dissolve in water but slightly soluble or insoluble in organic solvents.
Gentamicin quantification was done by using ninhydrin as a reaction compound [123]. The ninhydrin reacts with primary and secondary amines to give a purple color. These color changes can be studied calorimetrically. The gentamicin ninhydrin mixture gives linear relationship ranges from 1-80ug/mL at 400nm wavelength [124].

Table 1: Physical properties of gentamicin sulphate

<table>
<thead>
<tr>
<th>Physical properties of gentamicin sulphate</th>
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</thead>
<tbody>
<tr>
<td>Physical appearance</td>
</tr>
<tr>
<td>Solubility</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Chemical properties</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reaction with ninhydrin</td>
</tr>
</tbody>
</table>
1.10.4 Solvents:

Table 2: Solvents used for electrospinning

<table>
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<th>Solvents</th>
<th>Suppliers</th>
<th>Specifications</th>
<th>Item No</th>
</tr>
</thead>
<tbody>
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<td>BDH laboratory suppliers</td>
<td>99.8% purity</td>
<td>152834E</td>
</tr>
<tr>
<td>Dimethyl formamide (DMF)</td>
<td>Riedel-de Haen Germany</td>
<td>99% purity</td>
<td></td>
</tr>
<tr>
<td>Absolute Ethanol</td>
<td>SISCO research laboratory PVT, Mumbai India</td>
<td>99% purity</td>
<td>11526689</td>
</tr>
</tbody>
</table>

1.11 Scaffolds preparation equipment:

1.11.1 Electrospinning:

1.11.1.1 Setup-operating principles:

The electrospinning is a simple technology exists of four main components Figure (7): 1) high power supply 2) syringe pump 3) syringe needle with solutions 4) a collector for fiber deposition. The electric field is applied to the polymer solution where the positive pole is connected to the needle and negative electrode to the collector. Hence, when the voltage is applied the repulsive charge is accumulated at the tip of the needle which exist
in the form of a hemisphere[21]. The solution at the tip of the needle should possess high surface energy and surface tension within the electric field. When the repulsive charge overcomes the surface tension, this leads to the formation of Taylor cone. This will direct to the formation of polymer solution towards the negative electrode as a collector and produce fibers. These polymer solution leads to the evaporation of solvents, instability and finally, polymer solution is deposited on the collector as dry fibers ranging from nanometers to micrometers[22].

Figure 7: Electrospinning apparatus consists of a) flow meter for syringe pump b) power supply c) needle connected d) rotating collector drum e) chamber protect from the high voltage.
1.11.1.2 Electrospinning parameters:

There are many parameters control the electrospinning process; Solutions parameters and processing parameters. Solution parameter includes concentration of the polymer solution, molecular weight, viscosity, surface tension, solvents and conductivity/surface charge density. As well as processing parameters along with voltage, collector/needle distance, flow rate and the diameter of the syringe and also ambient parameters like humidity and temperature also play a major role in the fabrication of nanofibers for electrospinning process [125]. Customized electrospun fibers are invented by changing the parameters above [126].

1.11.2 Magnetron Sputtering:

Sputtering is simply called as momentum transfer between the particles Figure (8) [86]. Wherein, when a positive ion from sputtering gas with high-speed strike on a surface target of a material and surface atoms are ejected and deposited on the substrate [87][88]. Argon gas is commonly used as a sputtering gas. The ejected atoms are accumulated on the substrate via adjusting the distance between the target and substrate. The number of atoms exited from the surface per incident ion is known as sputtering yield ‘S’. S value depends on many parameters such as target material composition, experimental geometry, binding energy and properties of incident ions. And also experimental parameters such as voltage and current. In a conventional sputtering machine, the cathode is connected to the target and anode is connected to the substrate where the plasma in between them Figure (8).
Figure 8: Plasma sputtering technology
Figure 9: Schematic representation of sputtering technology [127]

The DC and RF sputtering process are mainly varied on the power supply installed. In DC sputtering process metals are only used as target for coating. However, in case of RF insulators are used as a target for coating purpose. This is mainly achieved by providing the RF potential to the target. In reality, when insulators are used as the target; their charges are accumulated on the surface of the target after striking of positive ions. This would make target surface inaccessible for the further bombardment of ions. Therefore, to inhibit this process both positive ions and electrons are bombarded directly to the insulator target [86]. It is achieved by applying RF power supply, it will lend enough energy for oscillating electron in the presence of alternating field to originate ionizing collision and a self-preserved discharge is maintained. However, Magnetron sputtering incorporation to DC/RF sputtering could enlarge the sputtering yield by applying a strong
magnetic field along the sputtering target for the confinement of plasma nearby target surface. In which, electric field $\overrightarrow{E}$ and magnetic field $\overrightarrow{B}$ are applied for electronic motion. Electron experience well-known Lorentz force in the magnetic field and an electric field force in electric field which leads to the circular motion of the electron nearby the target material and enhances the collision with the target and improves the sputtering yield. In sputtering process are take part in less vacuum because air contaminates such as oxygen or other gas impurities could create impurities deposition on the surface of the substrate and reduce plasma. Steps of plasma sputtering process is explained in Figure (9).

![Diagram of plasma sputtering process]

**Figure 10**: Factors depends on the plasma sputtering techniques.

### 1.12 Fiber preparation methods and electrospinning conditions:
1.12.1 Optimization of PVA and PCL individual fibers and multilayered structure.

**Polyvinyl alcohol (PVA):** 10 wt%/v% PVA was dissolved in deionized water and stirred for 3h at 80°C. The dissolved solution was electrospun to produce PVA fibers. Electrospinning parameters are voltage 15kV, distance 10cm, needle size 21G, and flow rate 1mL/h.

**Polycaprolactone (PCL):** 5.5 to 11 wt% of PCL was dissolved in 9:1 (wt%/wt%) CF:EtOH and stir for 1h. And also 8.5 to 12 (wt%) PCL solution was prepared in 1:1 (wt%/wt%) DMF:CF and stirred for overnight. Electrospinning parameters are voltage 7-15kV, distance 15cm, needle size 21G, and flow rate 1mL/h Table (4).

**Multilayered (PCL-PVA-PCL) electrospun fiber loaded with drug:** 10% (wt%/v%) PVA was dissolved in deionized water with 0mg/mL, 1mg/mL (1% wt/wt%), 2mg/mL (2% wt/wt%) and 4mg/mL (4% wt/wt%) of gentamicin sulphate (GS) and stir for overnight at 80°C. On the other hand, 11 wt% of PCL was prepared in 1:1 (wt%/wt%) DMF and CF and stirred for overnight. The electrospinning parameters are voltage 15kV, distance 15cm, needle size 21G, and flow rate 1mL/h, in which, the outer layers was electrospun for 2 hours each and middle layer loaded with drugs was electrospun for 1h respectively Table (6).

1.12.2 PCL sputter coated with Silver (Ag)

The PCL nanofibers were sputter coated with 99.9% of Silver target (Plasma Technology Limited 83 Tat Chee Avenue, Kowloon Tong, Hong Kong) and applied base pressure 1*10⁻³ Pa and working pressure 0.7Pa sputter coated for 30s, 60 and 120s. The applied voltage was 340-350 V and the current was 0.09A.
1.12.3 PCL-PVA-PCL sputter coated with Silver (Ag)

The PCL-PVA-PCL nanofibers with and without gentamicin sulphate were sputter coated with Ag with base pressure of $1 \times 10^{-3}$ Pa and working pressure 0.7Pa and coating for 30s. The applied voltage was 340-350 V and the current was 0.09A.

1.13 Characterization:

1.13.1 Scanning Electron Microscopy (SEM) and EDX.

The fiber morphology was characterized using the SEM images. The SEM images were obtained by using an SEM, LEO 1550 equipped with Schottky field emission gun (10 kV) and Robinson backscatter detector and FEI Quanta 200 scanning electron microscopy Figure (11). The all-fiber scaffolds were sputter coated with gold for 1min to minimize the charging effect. The entire fiber diameter was characterized by using imageJ an imaging processing software. All fiber diameters were measured and statistically analyzed.
The elemental composition of the fiber was measured by using Energy Dispersion X-ray spectroscopy (EDAX). EDAX was worked on SEM instrument in which the sample was bombarded with an electron beam where some electron collides with the specimen atoms own electrons and kicking them off in the process. A vacant is created inside the shell of the specimen, therefore, an electron from higher energy level is filled the vacant by emitting energy as X-rays [128]. Each element has its own X-ray emission range. The EDX tool was studied by using quanta 200 SEM Figure (11) under liquid nitrogen. The EDX tool was also referred for mapping the elemental composition in the samples.

Figure 11: FEI Quanta 200 scanning electron microscopy (SEM).
1.13.2 The Fourier Transform Infrared Spectroscopy (FT-IR)

The Fourier Transform Infrared Spectroscopy (FT-IR) was implemented to study the presence of the functional group in the scaffolds. FTIR used was Spectrum 400 FT-IR/FT-NIR Spectrometer (Perkin-Elmer) with wavelength ranges from 4000cm\(^{-1}\) to 400cm\(^{-1}\) Figure (12)

Figure 12: TGA FTIR set up (FT-IR Spectrometer Frontier/ TGA 4000 – Perkin Elmer)
1.13.3 Thermogravimetric analysis (TGA)

Thermogravimetric analysis (TGA) was done to study the heat resistant of the material with and without incorporation of drugs. TGA was measured on using Thermogravimetric Analyzer - Pyris 6 (Perkin-Elmer) Figure (13). 2-7mg of samples was taken and loaded on platinum for reading the weight loss at a rate of 10C/min from normal room temperature to 500C in a dry nitrogen environment.

Figure 13: Thermogravimetric Analyzer - Pyris 6 (Perkin-Elmer)
1.13.4 UV-VIS spectrophotometry:

UV-VIS spectrophotometry was used for the quantification of gentamicin sulphate release by using ninhydrin-gentamicin assay, which provide purple color and absorbance peak at 400nm. The instrument uses the ultraviolet-visible lights for the analysis of the chemical components concentration present. In absorption spectroscopy, light was absorbed by the sample solution and emit a specific amount of light. The absorbance of light is mainly due to the excitation of the electrons from ground state to excited state by the chemical components present in the sample. From this information; absorbance was measured to study the quantitative analysis of the colored compound present in the samples by comparing with the calibration curve. UV-Vis works in the electromagnetic spectrum from 190-750nm [129]. The UV-VIS spectroscopy used for the experiment was Agilent technologies cary 60 UV-Vis Figure (14) in Anti-doping Laboratory Qatar.

Figure 14: UV-VIS spectroscopy (Agilent technologies cary 60 UV-Vis)
1.13.5 Inductively coupled plasma mass spectrometry  ICP-MS:

Inductively coupled plasma mass spectrometry (ICP-MS) is a metal and non-metals at a concentration less than $10^{15}$ (part per quadrillion, ppq) on non-interfered low background isotopes. This method mainly works by ionizing the sample via inductive couple plasma and then the mass spectrometry categorizes ions based on their mass and quantify them. The ICP-MS was used to quantify the presence of Ag release in deionized water. It was done by using ICP-MS NexION 300D model Figure (15).

Figure 15: ICP-MS NexION 300D
1.14 In-vitro drug delivery of gentamicin Sulphate

1.14.1 Gentamicin-ninhydrin assay:

Gentamicin quantification was done by using ninhydrin (Biochemical Prod 440605V) as a reaction compound [123]. The ninhydrin reacts with primary and secondary amines to give a purple color. These color changes can be studied calorimetrically.

1.14.1.1 Preparation of Phosphate buffered solution (PBS):

One PBS tablets (MDL; MFCD00131855) was mixed with 200mL (as per the instruction on the PBS tablets bottle) of distilled water (pH 7.4) at room temperature to obtain 0.01 M phosphate buffer, 0.0027 M potassium chloride and 0.137 M sodium chloride and pH 7.4.

1.14.1.2 Preparation of Ninhydrin reagent:

Ninhydrin reagent (1.25wt/v%) was prepared by dissolving 0.0622g of ninhydrin in 5mL PBS solution (pH 7.4) to obtain concentration of 12.5mg/mL of ninhydrin solution. The mixture was vortexed for 10min followed by sonication for 30min. The prepared ninhydring should be consumed within 3 days if the solution was kept at room temperature.

1.14.1.3 Preparation of calibration curve:

The GS standard solution were prepared by serial dilution of 50mg/mL GS to obtain a ranges of 0-80ug/mL of gentamicin solution according to the Table (3) and used for the calibration curve Figure (16).
Table 3: Preparation of standard solutions protocol

<table>
<thead>
<tr>
<th>Final Concentration (µg/µl)</th>
<th>Amount A (µL)</th>
<th>A (µg/ml)</th>
<th>PBS (µL)</th>
<th>Vprep (µL)</th>
<th>V final (µL)</th>
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<tbody>
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</table>
Figure 16: Calibration curve for ninhydrin - gentamicin sulphate assay

1.14.1.4 Drug delivery assay of prepared scaffold:

The fiber scaffolds were weighted around 30-50mg and immersed in 7mL of PBS buffer solution (pH 7.4) and incubated at 37°C. And aliquot of 2mL of each sample at specific time interval 0.5, 1, 2, 3, 5, 24, 48, 144 and 168hrs was collected. And removed volume was replaced by 2mL PBS buffer solution to make same sink condition. The collected samples were stored at 4°C until analyzed.
Later on, Ninhydrin (2mg/mL): sample solutions (1.5:5) ratio was mixed together and heated in a water bath at 95°C for 30 min followed by cooling for 10 min. Finally, UV-VIS spectroscopy was measured under 400nm to find the release of GS during specific interval of time Figure (17).

Cumulative release of drug was calculated by using following equation 1.

Cumulative drug release % : \( = \frac{M_t}{M_\infty} \times 100 \) \hspace{1cm} (1)

Where \( M_t \) was the amount of GS released at time (t) whereas \( M_\infty \) the amount of GS added to electrospinning scaffold:

Figure 17: Schematic procedure to study the drug release
1.14.1.5 Encapsulation efficiency:

Firstly 5-8mg of electrospun scaffolds was mixed with 1 mL of chloroform and vortexed for 5min until the PCL layer mix well. Henceforth, 1mL of PBS (pH 7.4) was added to the solution and mix for 30min by shaking at 1000rpm. Hereafter, the solution mixture was centrifuge at 6000rpm for 5min. Then, 800ul of supernatant was collected and blended with 240uL of ninhydrin solution (2mg/mL) (1.5:5 rations). Then for the complex formation of gentamicin-ninhydrin the solution was heat at 95°C for 30min and later on to stop the ninhydrin-gentamicin sulphate reaction the solution was cooled for 10min. Subsequently, UV-VIS absorbance was measurement at 400nm for the solution. The gentamicin concentration in the scaffold was calculated from the calibration curve. Encapsulation efficiency was calculated by using the following Equation (2).

Encapsulation efficiency % = \( \frac{\text{Actual gentamicin loaded in scaffolds}}{\text{Theoretical gentamicin concentration}} \times 100 \)  

(2)

1.15 In vitro release of Ag:

In vitro release of Ag was carried out as in Figure (18). 7-10mg of the PCL-PVA-PCL/Ag fiber scaffold in a 15mL test tube and 5 mL of deionized water were added to the test tube. Incubate the test-tube in water-bath at 37°C. At each interval of time, collect 1 ml of the deionized water in the test tube and refill with 1ml to establish same sink conditions. Finally, to validate the amount in parts per billion (ppb) of silver present in the solution was studied by using ICP-OES.
1.16 **In-vitro bio-compatibility studies:**

1.16.1 **Cell lines:**

Human keratinocytes, HaCaT cell line was obtained from Cell Line Services (Germany) and human fibroblasts, Wi38 cell line was obtained from American Type Culture Collection (USA). Cells were grown in DMEM (Dulbecco's Modified Eagle's medium) supplemented with 10% fetal bovine serum (FBS, SIGMA, Germany) and 1% Penicillin/Streptomycin (SIGMA, Germany) at 37°C in a humidified atmosphere containing 5% CO₂. Cells were passaged when they reached confluence of 70% and used for the experiments.
1.16.2 Cell seeding

HaCaT and Wi38 cells were seeded on 96well plate (Nunc 96-Well) at a cell density of 7000 and 14000 cells respectively in 200μL of the media. After 24h cells were treated with scaffolds. The samples were cut into small square cubes with 0.5cm x 0.5cm by using ruler and scissors and then sterilized under UV for 15min each side. The sterilized scaffolds were added to the 96 well plates and incubated for 48h and 72h respectively.

1.16.3 In-vitro quantitative cell viability assay:

Sterilized square shaped sample were added to Wi38 and HaCaT in 96 well plate with density of 14000 and 7000cells/well. Afterwards, 96well plates was incubated for 48h and 72h in a humidified atmosphere of 5% of CO2. Followed by incubation for subsequent period the viability test was accessed by Alamar blue assay. In alamar blue assay, add 20ul of alamar blue (7-hydroxy-10-oxidophenoxazin-10-ium-3-one,[130] were added to the cells and incubated for 2h in a humidified atmosphere of 5% of CO2. The fluorescent was measured in Tecan multimode reader M200 Pro at excitation between 570nm and an emission at 590 nm respectively Figure (19).
Figure 19: Schematic representation of In-vitro cell toxicity test
1.17 Antibacterial Studies:

1.17.1 Medium preparation:

Luria broth (LB) medium was prepared by taking 28g of nutrient agar (HiMedia laboratories Pvt.Ltd. M001-500G) was mixed with 1L of distilled water. The prepared mixture was autoclaved at 121°C for 1h. Followed by sterilization the LB agar medium was pour into the pteridishes to cool down and solidify. All the above procedure was done under laminar to avoid contamination.

Nutrient broth (NB) medium was processed by suspending 25g in 1000 ml purified/distilled water. The prepared mixture was heated autoclaved at 121°C for 1h[131]. The NB medium was used for the preparation of bacterial suspension incorporation with the samples.

Phosphate saline solution was prepared by mixing 0.91% NaCl in distilled water.

1.17.2 Antibacterial testing and spreading the plates.

The antibacterial testing was carried out for the PCL only, PCL fibers sputter coated with Ag at 30s, 60s and 120s sputtering time, multilayered (ML) structure of PCL-PVA-PCL electro spun fibers incorporated with gentamicin sulphate as well as sputter coated with Ag. They are labeled as PCL only, PCL/Ag 30s, PCL/Ag 60s, PCL/Ag 120s, ML only, ML/GS 1%, ML/GS4%, ML /Ag 30s and ML/GS/Ag respectively with 3 repetition to evaluate standard error.
Firstly, the bacterial was distributed all along the medium via streaking technique. In which the sterile loop was used to collect bacterial colonies from streak plated petridish to a PBS solution.

Secondly, the samples were cut into small cubes with size 1.5x1cm with ruler and scissors. The scaffold samples were sterilized under UV for 30min both sides. Later on, the cubes was placed in the 2mL of bacterial suspension of gram positive *Bacillus pumilus* and gram negative *E.coli* in 50mL centrifuge tube. And the centrifuge tube was incubated at temperature of 28°C and shaking at 100rpm respectively. After each interval of time 2h and 6h the 10uL of suspension was mixed with 990uL of sterile saline solution (B) in an eppendorf tube. And followed by, pipette 100uL of bacterial suspension from B to the appropriate labeled petri-dishes and spread by using spreader for uniform distribution of colonies. Subsequently, the petridishes was incubated at 37°C for 24h. And picture was obtained and number of colonies was counted. The whole process was explained in figure shown below Figure (20).
Figure 20: Schematic procedure for antibacterial testing
RESULTS AND DISCUSSIONS

1.18 Optimization of PVA and PCL individual fibers, and multilayered structure.

1.18.1 Poly (vinyl alcohol) PVA:

PVA 10wt%/v% solution was electrospun to prepare nanofibrous mats, where the electrospinning parameters applied are voltage 15kV, flow rate of 1ml/h, and needle tip to collector distance of 10 cm. Scanning electron microscope of the electrospun nanofibers are given in the Figure (21). A beadles uniform fibers with an average diameter of 107.25±21.07 nm is obtained.

![SEM image](image1)

Figure 21: a) SEM image of 10wt%/v% PVA (electrospinning parameters are applied voltage 15kV, flow rate of 1ml/h, and needle tip to collector distance is 10 cm) (magnification: 1μm). b) Normal distribution of fiber diameter
1.18.2 Polycaprolactone (PCL):

PCL solution was prepared in 1:1 (wt/wt%) DMF:CF at different weight percentage of 8.5wt%, 10wt%, 11wt% and 12wt% respectively. The electrospinning parameters are described in Table (4). SEM results show that, at 8.5wt% and 10wt% of PCL, it has shown some beads on the scaffold structure. However, at 11wt% and 12wt% uniform fiber with diameter around 407.04±112.1nm and 901.14±350nm respectively Figure (22).

The optimization of polycaprolactone was done by using different solvents at various weight percentages to obtain uniform fibers with smaller diameters Table (4). PCL is a hydrophobic polymer which is easily soluble in chloroform. However, chloroform has lower electric conductivity [42]. As a result, insulating polymer solution could not beat the surface tension in high electrostatic force. Therefore, introduction of conductive polymer solution is essential for improved electrospinning process. So, DMF show moderate conductivity due to its dielectric constant [132].

There is critical minimum concentration (Ce) required for the formation of beaded fibers. The concentration above Ce shows a reduction in the beads as well as increase in the fiber diameter and uniformity. Concentrations lower than Ce shows drops like electrospaying. The value of Ce depends on polymer molecular weight, solution and chemical properties of the polymer. Polymer solvent plays an important role in selecting proper Ce and also morphology of the fiber. Hence, selection of solvent is an important factor for better fiber production in electrospinning process. Therefore, the critical concentration for 1:1 wt%/wt% of DMF: CF is 10% of PCL Figure (22). 11wt% of PCL was used for the further production of electrospun fibers due to more uniform beadles fiber with average diameter of 407.04±112nm Figure (22e).
Table 4: Electrospinning parameters applied for optimization of PCL fibers.

<table>
<thead>
<tr>
<th>Weight % of the PCL (wt/wt%)</th>
<th>Solvents ratio (wt/wt)</th>
<th>Flow rate (ml/h)</th>
<th>Distance (cm)</th>
<th>Voltage (kV)</th>
<th>Needle size (G)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 8.5%</td>
<td>1:1 DMF:CF</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>21G</td>
</tr>
<tr>
<td>2 10%</td>
<td>1:1 DMF:CF</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>21G</td>
</tr>
<tr>
<td>3 11%</td>
<td>1:1 DMF:CF</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>21G</td>
</tr>
<tr>
<td>4 12%</td>
<td>1:1 DMF:CF</td>
<td>1</td>
<td>15</td>
<td>15</td>
<td>21G</td>
</tr>
</tbody>
</table>

Table 5: Average fiber diameter of the prepared PCL mats in different weight percentage.

<table>
<thead>
<tr>
<th>Weight percentage of PCL</th>
<th>Average fiber diameter (nm)</th>
<th>Beads/uniform</th>
</tr>
</thead>
<tbody>
<tr>
<td>8.5% PCL 1:1 CF:DMF</td>
<td>218.2±100.6</td>
<td>Beads/not uniform</td>
</tr>
<tr>
<td>10% PCL 1:1 CF: DMF</td>
<td>365.5±144.5</td>
<td>Beads/not uniform</td>
</tr>
<tr>
<td>11% PCL 1:1 CF: DMF</td>
<td>407.0±112.1</td>
<td>No beads/uniform</td>
</tr>
<tr>
<td>12% PCL 1:1 CF: DMF</td>
<td>901.1±350.5</td>
<td>No beads/uniform</td>
</tr>
</tbody>
</table>
Figure 22: SEM images of electrospun PCL in 1:1 (wt/wt%) DMF:CF at different weight percentage of PCL a) 8.5wt%PCL, b)10wt%PCL, c) 11wt%PCL, d) 12wt%PCL e) Average fiber diameter and f) Fiber diameter distribution of 11wt%PCL (magnification 10μm).

1.19 PCL coated with Ag

The individual PCL fibers was sputter coated with silver at different sputtering time 30s, 60s and 120s with base pressure of 1x10^{-3} pa, working pressure 0.7Pa, voltage 340-350 V and the current was 0.09A. The prepared electrospun sputter coated fibers are labeled as PCL only, PCL/Ag 30s , PCL/ Ag 60s and PCL/ Ag 120s.

The morphology of the fibers was characterized by SEM, elemental analysis by EDX and XRD. Moreover, cytotoxicity and antibacterial properties was studied for the optimized
coating time for the further preparation of multilayered electrospun fibers loaded with drug and sputter coated with Ag for the biomedical application.

1.19.1 Scanning Electron Microscope (SEM) & EDX:

The SEM, EDX and mapping of Ag in electrospun PCL fibers was shown in Figure (23). As mentioned in previous section, 11wt% of PCL was used for the preparation of electrospun fibers. The SEM images at Figure (23a), (23b) and (23c) demonstrate a uniform distributed beadles fibers. As shown in the EDX peaks in Figure (23a), (23b) and (23c) the Ag has increased for 30s, 60s and 120s with a weight percentage of Ag 1.65, 3.58 and 4.58 % respectively. Moreover, the mapping has shown a clear distribution of Ag at different sputtering time for sequential coating time. However, the presence of aluminum peaks was shown on each EDX peaks because sample fiber was held by the aluminum foil for getting proper surface for EDX characterization.
Figure 23: SEM images of PCL coated with Ag a) PCL/Ag 30s  b) PCL/Ag 60s and c) PCL/Ag 120s (Magnification 10μm)
1.19.2 X-ray diffraction (XRD).

The XRD of PCL sputter coated with Ag at different sputter time is shown in Figure (24). The peaks at 21.25° and 23.42° related to PCL fibers. Contrarily, there are four various peaks noted at 38.46, 44.52, 64.72 and 77.48 for PCL/Ag 30s, 38.31, 44.44, 64.75, 77.6 for PCL/Ag 60s and 38.15, 44.51, 64.64 and 77.64 for PCL/Ag 120s respectively. The illustrated peaks demonstrate for face centered cubic structure of Ag phase with reflection planes at (111), (200), (220) and (311) respectively [133].

Figure 24: XRD spectra for electrospun PCL sputter coated with Ag at different time a) PCL peaks b) Indexing the presence of silver in PCL only, PCL/Ag 30s, PCL/Ag 60s and PCL/Ag 120s.
1.19.3 Cytotoxicity of Ag:

The cytotoxicity of the electrospun nanofibers was studied by using Wi38 and HaCaT cell line for as PCL, PCL/Ag 30s, PCL/Ag 60s and PCL/Ag 120s for 48 hours and 72 hours Figure (25). In the case of Wi38 Figure (25a), at 48h of incubation shows toxicity affect at the PCL at 120s coatings compared with PCL as control. As time passes, the silver molecules are released at it exhibit toxic effect for the 72h of incubation. Because PCL/Ag 30s, PCL Ag/60s and PCL/Ag 120s has shown p<0.05 compared with PCL as control. However, PCL fiber displayed some reduction in the cell growth, these could be due to the solvent present in the electrospun fiber PCL such as DMF and CF. But there is a clear dependency on toxicity versus amount of silver present in the electrospun PCL fiber. PCL coated for 30s shows lower toxic effect as compared to other in the case of Wi38.

In the case of HaCaT cell line Figure (25b), after 48h there is a reduction in the cell growth in all silver coated samples. Yet, Silver didn’t inhibit the growth of cell after 72h in the case of PCL/Ag 30s, PCL/Ag 60s and PCL/Ag 120s. As the sputtering time increase toxicity has increased for all three samples, at p<0.05 for all three samples after 48h and 72h in comparing PCL only as control. The same solvent effect has shown few reduction growth of cell line in the case of HaCaT for PCL only from the control. Finally, in summary it can be concluded that, the samples sputter coated either 30s or 60s is the best compared with 120s coating time. Therefore, next step was the optimization of antibacterial properties and choose the best antibacterial coating time for biomedical application such as wound healing.
Figure 25: In vitro cell viability test of a) Wi38 b) HaCaT cell line PCL, PCL/Ag 30s, PCL/Ag 60s and PCL/Ag 120s (n=5). ***p<0.05.

1.19.4 Antibacterial study:

The antibacterial properties of the PCL only, PCL/Ag 30s, PCL/Ag 60s and PCL/Ag 120s were carried out with two kinds of microorganism: *Bacillus pumilus* (Gram positive). The release of Ag from the sample with respect to time of incubation for 2h and 6h was studied. The results were summarized in Figure (26) and Figure (27). In Figure (26) it clearly demonstrate that, the presence of silver has reduced the growth of bacterial colonies. The Figure (26) was plotted graphically in Figure (27). The PCL fiber itself has never shown any antibacterial properties. However, in the case of PCL/Ag 30s there was not any antibacterial character during first 2h in gram positive, but has improved the
antibacterial activity after 6h of incubation by 96% from the control for gram positive. The PCL/Ag 60s has exhibited antibacterial inhibition from 1.85% to 95% for *Bacillus pumilus* for 2h and 6h incubation respectively. Moreover, PCL/Ag 120s displayed antibacterial inhibition from 48% to 92.45% for 2h and 6h incubation respectively. From these results we can conclude that, for gram positive bacteria as the time passes the release of Ag inhibit the growth. In addition, the sputtering time has not shown much change in the antibacterial properties. Only difference is in the release of silver to the media. Statistically, after 6h of incubation has shown much more variation in antibacterial inhibition compared with PCL as control (p<0.05).

The antibacterial properties of silver are by various methods [134], [135]. For instance, the physical contact of silver particles to the cellular membrane of the bacteria could leafs to cellular damage, silver ion interact with cellular activity and DNA replication and Inhibit the growth of bacterial cells [136]. These results depict that the release of silver coated on the surface of electrospun fiber mats has exhibited antibacterial inhibition with time. Therefore, this type of coating could provide a better antibacterial inhibition for long term for biomedical application such as wound healing process. Hence, PCL sputter coated with Ag for 30s and 60s was more precise in antibacterial properties and cell cytotoxicity. Therefore, multilayered structure was sputter coated for 30s for better antibacterial and reduced cell cytotoxicity.
Figure 26: Antibacterial activities of the electrospun fibers coated with Ag against gram positive *Bacillus pumilus* (a) Control, b) PCL only, c) PCL/Ag 30s, d) PCL/Ag 60s and e) PCL/Ag 120s and also i) row *Bacillus pumilus* after 2h of incubation and ii) 6h of incubation.

Figure 27: Antibacterial activities of electrospun PCL sputter coated with Ag with a) number of colonies (CFU/mL) Ag and b) antibacterial inhibition (%) was plotted for *Bacillus pumilus*. 
1.20 Multilayer PCL-PVA-PCL loaded with GS and sputter coated with Ag:

The multilayer PVA/PCL loaded with hydrophilic gentamicin sulphate was prepared by optimized condition. Various drug concentrations loaded around 1mg/mL, 2mg/mL and 4mg/mL was prepared on multilayered electrospun fiber scaffolds. Later on, 1mg/mL of scaffold was sputter coated with Ag. The fiber characterization was studied by SEM, FTIR, DSC and water contact angle measurement. Moreover, in-vitro release of gentamicin from the multilayered electrospun fiber scaffolds was considered. Furthermore, in-vitro cell toxicity of the electrospun fiber MATS was investigated by using Wi-38 and HaCaT human cell line. Antibacterial inhibition (%) was also investigated with gram negative and positive bacteria.

1.20.1 Fiber characterization:

1.20.1.1 Scanning Electron Microscope (SEM) :

Multilayered PCL-PVA-PCL was prepared by using 11wt% of PCL solution and 10wt% PVA solution in 1:1 DMF:CF and deionized water respectively. The electrospinning condition was shown in Table (6). The SEM images showed that, the fibers are uniformly distributed with fiber diameter 407.0±112.1 Figure (28a) and 107.25±21.07 Figure (28b) for PCL (outer layer) and PVA (middle layer) respectively
Table 6: Electrospinning condition for multilayered PCL-PVA-PCL.

<table>
<thead>
<tr>
<th>Polymer layer</th>
<th>Voltage</th>
<th>Distance</th>
<th>Flow rate</th>
<th>Time (h)</th>
<th>Needle size</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL</td>
<td>15</td>
<td>15cm</td>
<td>1</td>
<td>2</td>
<td>21G</td>
</tr>
<tr>
<td>PVA (0,1,2,4 mg/mL of GS)</td>
<td>15</td>
<td>15cm</td>
<td>1</td>
<td>1</td>
<td>21G</td>
</tr>
<tr>
<td>PCL</td>
<td>15</td>
<td>15cm</td>
<td>1</td>
<td>2</td>
<td>21G</td>
</tr>
</tbody>
</table>

Figure 28: SEM images of a) PCL outer layer b) PVA loaded with drug (middle layer) c) PCL fiber (outer layer)

1.20.1.2 Fourier-transform infrared spectroscopy (FTIR):

FTIR was characterized for powder of pure GS, pure PCL, pure PVA, 0 wt% GS ML, 1 wt% GS ML, 2 wt% GS ML and 4 wt% GS ML respectively Figure (29).
In Figure (29), FTIR spectrum of Gentamicin sulphate show a broad peak at 3000 cm\(^{-1}\) due to the presence of O-H and N-H bonds. The typical bands at 1644, 1633 and 1360 cm\(^{-1}\) represent the presence of amide I, amide II and amide III bonds of gentamicin sulphate respectively. Peaks notified at 1060 cm\(^{-1}\) is due to HSO\(_4\). The peak at 612.3 cm\(^{-1}\) represent the presence of SO\(_2\) band. On the other hand, FTIR of PCL exhibit peaks at 2959 cm\(^{-1}\) (asymmetric CH\(_2\) bond), 2921 cm\(^{-1}\) (symmetric CH\(_2\) bond), 1732 cm\(^{-1}\) (carbonyl bond),
bond), 1290 cm\(^{-1}\) (C-O bond) and 1240 cm\(^{-1}\) (C-O-C asymmetric bond). Also the PVA show typical bands such as at 3374 cm\(^{-1}\) (hydroxyl bond), 2959 cm\(^{-1}\) (asymmetric CH2 bond), 2921 cm\(^{-1}\) (C-H bond), and 1472 and 1105 cm\(^{-1}\) (C-O bond). The same type of peaks was observed for PCL and PVA fibers are already reported [62], [137].

The FTIR show a broad peak of 3374 cm\(^{-1}\) (hydroxyl bond) demonstrates the presence of GS as well as PVA. On the other hand, due to low concentration of gentamicin most of its peak was difficult to analyze by FTIR. In Figure (29), the multilayer structure demonstrates the peaks of PCL only because FTIR can only penetrate till 2um. Hence, in multilayer structure the outer layer is thicker than these limits. Therefore, it was not possible to show the peaks of PVA and GS.

1.20.1.3 Thermogravimetric analysis (TGA):

Figure (30) demonstrate the TGA results of pure PCL, pure PVA, PCL-PVA-PCL (multilayered electrospun mats loaded with 1wt% GS, 2wt% GS and 4wt% GS respectively. The pure PCL nanofibers has shown one step degradation at 320\(^{\circ}\)C and completely degraded at 460\(^{\circ}\)C. The pure PVA decomposes in 3 steps. Firstly, from as shown Figure (29) 50–170 °C is due to the moisture vaporization. Followed by, weight loss from 250 °C is the decomposition of PVA side chain. Finally, further decomposition of polyene residues take place which results in carbon and hydrocarbons.

The incorporation of PVA-PCL composite electrospun fiber mats reduced its thermal stability as compare to PCL. However, it improved in the case of pure PVA. PVA-PCL loaded with 0wt% and 1wt% of GS has shown the similar thermal degradation. But improved its thermal stability via increasing the weight percentage of drug loaded in the
electrospun fiber mats. Therefore, 2wt% and 4wt% GS ML has shown increase in thermal stability. This would be due to the crystallinity of the drug molecules. This same type of results was noted in literature [138].

Figure 30: Comparative TGA results of all the prepared fiber samples. Including pure PCL, pure PVA, PCL-PVA-PCL multilayered electrospun mats loaded with 1wt% GS, 2wt% GS and 4wt% GS respectively.
1.20.2 Encapsulation efficiency of the GS in the fiber scaffolds:

The encapsulation efficiency of the Gentamicin sulphate in the fiber scaffolds was calculated Figure (31) and results show that, multilayered scaffolds of 1wt%, 2wt% and 4wt% of gentamicin sulphate have encapsulation efficiency of 54.8±22.9%, 54.4±13.46% and 64.39±13.99% respectively. This is due to the fact that, PVA loaded with GS is electrospun separately in multilayered therefore, there is chance for GS accumulation in the syringe and reduction in the encapsulation efficiency. On the other hand, as the drug concentration increases in the multilayered structure its encapsulation efficiency is improved in the case of 4wt% of GS in multilayered structure.

![Encapsulation Efficiency Graph](image)

Figure 31: Encapsulation efficiency of the GS in the fiber scaffolds
1.20.3 In-vitro release of drug.

The in vitro release of GS from the electrospun fiber mats was studied using ninhydrin-gentamicin assay. The set of standards was prepared from gentamicin sulphate with ranging concentrations of 0 to 80ug/mL according to the Table (3). The calibration curve is shown in Figure (16) with regression 0.9997. The calibration curve was used to analyses the concentration of gentamicin sulphate release from the fiber scaffold into the PBS medium at each interval of time.

After plotting the calibration curve the drug release of the scaffold was studied for multilayered structure for 7 days. The cumulative drug release per mg of scaffold was investigated with taking the percentage of drug present in solution at particular interval of time with total encapsulated drug from encapsulation efficiency. The results are shown in Figure (32). Multilayered has shown initial burst release over 5h was around 65%. After initial burst release its shown slower release of drug over 7 days where only 80% of loaded drug was released. In the case of multilayered structure, there are many factors contribute for the sustained release of drug, they are thickness of the fiber, amount of drug loaded, fiber diameters and porosity [27][139]. The initial burst release suggests that a good amount of GS was released during first 5h. Succeeding initial burst, the outer layer of multilayer scaffold will swells and stabilize and trap the drug from further release. These phenomena makes the continuous and sustained release of GS in ML for over 7 days where only 79% was released [27].

The different weight percentage of GS was loaded into the fiber scaffolds and studied release kinetics. The results showed that, 1wt% GS ML has showed 65% of intial release of drug from the scaffold. Also, in the 2wt% GS ML has only 53% of drug initial burst
release during first 5 hours. Formerly, showed a slow and sustained release with only 60% was release over 7 days. But in the case of 4wt% GS ML it has shown that, only 40% of drug released during first 5 hr and showed sustained and slow release for 7 days. As the amount of GS increases the initial burst release of the drug will be reduced in accordance with swelling and stabilizing of the scaffold structure. However, over all amount of release of gentamicin from scaffold is shown in Figure (32b) where amount of drug loaded contribute for the results here where, multilayered 4wt% > multilayered 2wt% > multilayered 1wt% in this order. From the release profile it could be concluded that it is solution-diffusion mechanism because from the graph compared to (Figure 32).

Figure 32: cumulative release of GS from electrospun scaffolds a) cumulative % of drug loaded 1, 2, 4 wt% in multilayered structure b) Cumulative release of GS per mg of scaffolds of core shell, and multilayered structure.

multilayered is suitable candidate for controlled release of drug. But, if the hydrophilic core was incorporated with the hydrophobic shell with porous structure would leads to
initial burst release in the drug delivery. But this issue could be managed by introducing multilayered electrospun fiber would leads reduce the initial burst release and contribute for sustained release of drug via solution-diffusion mechanism.

1.20.4 In-vitro release of silver.

In-vitro release Ag from ML 1wt%GS/Ag 30s was studied by incubating the fiber mats in de-ionized water for 70h. The result exhibits Figure (33) show a smooth release of Ag for over 70h from fiber scaffolds surface. There was a smooth release in ppb per mass of the scaffold. So these data suggests that this type of scaffold should provide sustained release of Ag for prolonged time, which is suitable candidate for anti-bacterial property for bio-medical application such as wound dressing.

![Figure 33: cumulative release of Ag (ppb/mg of the fiber scaffold) for PCL/Ag 30s, PCL/Ag 60s, PCL/Ag 120s and PCL/Ag 240s respectively.](image)
1.20.5 Cytotoxicity of the fibers:

The cytotoxicity of the electrospun nanofibers was studied by using Wi38 and HaCaat cell line for with and without gentamicin sulphate loaded multilayered (ML) electrospun fiber mats which is labeled as 0wt% GS ML, 1wt% GS ML and 4wt% GS ML for 72hours Figure (34). In the case of Wi38, 0wt% GS ML has shown cell viability of 88% for core-shell structure and more than 101% for multilayered structure for 48h. moreover, 97% and 107% for multilayered structure without loaded with GS respectively. However, incorporation of GS to the drug to the scaffold had some toxic effect on the viability of cell growth. For instance, 1mg/mL multilayered and 4mg/mL multilayered have cell toxicity around 19%, 25% and 34% respectively.

In the case of HaCat, same effect has been noted where increase in the drug has minor toxic effect on the viability of cells. After 72h there is a higher reduction in the growth of cells compared with the control. These could confirm the toxic effect created on cell line (Wi38 and HaCat) by gentamicin sulphate. And also, the core shell and multilayered fiber scaffold demonstrate similar cell toxicity because the components in the material is same only variation in the structure of the fiber scaffolds. These results conclude that, as the concentration of the drug increases, it shows a toxicity effect towards the keratinocyte (HaCat) and fibroblast (Wi38) cell line.
Figure 34: In vitro cell viability test of a) Wi38 b) HaCat cell line in pristine PVA/PCL core shell without drug and 1mg/mL of GS, multilayered fiber scaffold without and with GS (1mg/mL and 4mg/mL) (n=5). **p<0.05.

### 1.20.6 Antibacterial Study:

The antibacterial properties of the electrospun multilayered fibers loaded with gentamicin sulphate and silver was carried out two kinds of microorganism: *Bacillus pumilus* (Gram positive) and *Escherichia coli* (Gram negative). They are labeled as ML 0% GS, ML 1% GS, ML 4% GS, ML Ag and ML 1%GS/Ag. The results were summarized in Figure (35) and Figure (36) for gram positive *Bacillus pumilus* and Figure (37) and Figure (38) for gram negative *E.coli*. The presence of gentamicin sulphate in the middle layer of multilayered electrospun fibers make it controlled release and sustain the antibacterial activity. Gentamicin is a bacteriocidal antibiotics which intrude the protein synthesis by binding irreversibly with the 30s subunits of ribosomes. These process is alike with other aminoglycosides. Gentamicin are active against many bacterial infection caused mainly by gram negative such as *Pseudomonas, Proteus, Escherichia coli, Klebsiella pneumoniae, Enterobacter aerogenes, Serratia*, and the Gram-positive *Staphylococcus*
The results from Figure (36b) show that multilayered fiber PCL-PVA-PCL itself showing antibacterial activity around (11.1% and 35.7%) during 2h and 6h incubation respectively with the control. However, 1%GS loaded samples has showed antibacterial inhibition 42.59% during 2h of incubation and it got reduced to 26.78% after 6h of incubation with respect to control. This is due to the initial burst release of gentamicin sulphate but it later on it was insufficient to kill the bacteria inside the drug. But the presence of 4%GS has showed around 45.2% during first 2h of incubation but improved to 99.51% by 6h of incubation. Finally, in ML Ag and ML 1%GS Ag the antibacterial efficiency has improved from 44.44% to 99.51% in ML Ag and 48.14 to 93.76% in ML 1%GS/Ag. Statically, for ML Ag and ML 1%GS/Ag after 6h of incubation has shown much more variation in antibacterial inhibition compared with control (p<0.05).
Figure 35: Antibacterial activities of the multilayered electrospun fiber mats loaded with drug and sputter coated with Ag against gram positive *Bacillus pumilus* with column (a) Control, b) ML 0% GS, c) ML 1% GS, d) ML Ag and e) ML 1% GS/Ag and also i) row *Bacillus pumilus* after 2h of incubation and ii) 6h of incubation.

Figure 36: Antibacterial activities of multilayered electrospun fiber mats loaded with drug and sputter coated with Ag against gram positive *Bacillus pumilus* a) number of colonies (CFU/mL) b) antibacterial inhibition (%)
On the other hand in the case Figure (37) & Figure (38) gram negative bacteria (*E.coli*), ML 0%GS (11.7%), ML 1%GS (33.85%), ML 4%GS (54.44%), ML Ag (63.47%) and ML 1%GS/Ag (53.71%) has shown antibacterial inhibition (%) for first 2h of incubation. However, after 6h of incubation the ML 0%GS (34.97%), ML 1%GS (30.98%), ML 4%GS (90.33%), ML Ag (82.49%) and ML 1%GS/Ag (71.39%) antibacterial inhibition has been observed.

These results recommend that, multilayered structure loaded with gentamicin sulphate sputter coated with Ag has improved the antibacterial properties of the material. Moreover, the samples antibacterial properties could be improved by addition of antibacterial drug into the samples. And it can give long antibacterial by reducing the initial burst release of the drug as well as consistent release of Ag from the surface of the electrospun fiber.
Figure 37: Antibacterial activities of the multilayered electrospun fiber mats loaded with drug and sputter coated with Ag against gram negative *E. coli* with column (a) Control, b) ML 0% GS, c) ML 1% GS, d) ML Ag and e) ML 1%GS/Ag and also i) row after 2h of incubation and ii) 6h of incubation.

Figure 38: Antibacterial activities of multilayered electrospun fiber mats loaded with drug and sputter coated with Ag against gram negative *E. coli* a) number of colonies (CFU/mL) b) antibacterial inhibition (%)
CONCLUSION

In this work preparation and characterization of multilayed fiber electrospun fiber mats of PVA/PCL polymers loaded with gentamicin sulphate was studied. Uniform fibers were prepared by electrospinning process. In-vitro release of GS suggest that, multilayered fiber mats reduces initial burst release of drug from the scaffolds. Moreover, initial burst release in multilayer could be reduced by improving the amount of loaded GS in the scaffolds. The cytotoxicity study illustrate conclude that, there is a toxic effect for the increase in the drug concentration for Wi38 and Hacaat cell line. However, the component PCL and PVA is biocompatible because it has improved the growth of cells. On the other hand PCL was sputter coated with Ag to investigate the in-vitro release of Ag and cytotoxicity of the scaffolds. It is summarized that, Ag coated scaffolds have toxicity effect towards the cell line. However, it possess a gradual release of Ag for the antibacterial activity. Antibacterial inhibition (%) has improved with the addition of drug as well as silver particles to the surface of the outer layer of the electrospun fiber. Therefore, it is concluded that, the prepared fiber scaffold could be promising material for sustained protection from antibacterial for wound dressing applications.
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