BIO-ACTIVE ANTIMECROBIAL SILVER-CHITOSAN COMPOSITE COATING ON 316L STAINLESS STEEL BY ELECTROPHORETIC DEPOSITION METHOD

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IN

BIOMEDICAL ENGINEERING

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CERTIFICATE

This is to certify that the thesisentitled**"BIO-ACTIVE ANTIMICROBIAL SILVER-CHITOSAN COMPOSITE COATING ON316L STAINLESS STEEL BYELECTROPHORETIC DEPOSITION METHOD"** submitted by **Avinash Dung Dung (110BM0639)** towards the partial fulfilment of therequirement for the degree of Bachelorof Technology in Biomedical Engineering from Departmentof Biotechnology & Medical Engineering with specialization in "Biomedical Engineering" at National Institute of Technology Rourkela is an authentic work carried out by him under my guidance and supervision**.**To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/ Institute for the award of any Degree or Diploma.

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ABBREVIATIONS

316LSS = 316L Stainless Steel

EPD = Electrophoretic deposition

FESEM = Field emission scanning electron microscope

g = Gram

min = Minute

V = Volts

%wt = weight percentage

XRD = X-ray diffraction

Ag= Silver

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ABSTRACT

316L Stainless steel is used extensively as medical implants which also includes pins, screws and orthopedic implants like total hip and knee replacements. So it would be beneficial to apply antimicrobial coating on 316L Stainless steel to minimize the risk of infection from open wounds and surgical conditions. In this research Silver-Chitosan composite was coated on 316L Stainless steel sample to make it an antimicrobial surface. Silver has been used as an antibacterial agent due to its strong inhibiting effect on bacteria. Silver has the properties like to obtain desired shape and size , easy handling for surface modification and ability of silver ions torelease themselves efficiently. Silver also has the added benefit of being less toxic to human tissues than harmful microorganisms. Chitosan is used along with silver to induce more antibacterial property of the biomaterial. Application of chitosan is due to its biodegradability, biocompatibility and non-toxic to human tissues. Also Chitosan exhibits an antibacterial activity to both Gram-positive and Gram-negative bacterial strains. Due to these unique properties, Chitosan and its derivatives are used as biomaterials in medicine, pharmaceuticals and food industries. In this project Silver-Chitosan coating on 316L Stainless steel by electrophoretic deposition was done to study the antibacterial property of Silver-Chitosan coating. First Silver was deposited on 316L Stainless steel at different supply voltages and at different deposition time. Then the prepared Silver coated samples were characterized according to their surface morphology and the samples on which silver was deposited in particulate form were chosen for chitosan coating. Then Chitosan was deposited on the chosen samples by electrophoretic deposition method. The prepared samples were then characterized for their surface morphology by FESEM technique and optical microscopy. The crystallinity of the samples were determined by XRD spectroscopy. The samples were then kept in a petri plate containing agar medium and the samples were exposed to E.Coli bacteria. The petri plate was then kept in an incubator overnight and the antibacterial property of the samples were studied.

KEYWORDS:- 316L SS, Silver, Chitosan, antibacterial, XRD, FESEM, E.Coli

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

Bacterial infections cases have increased in today's civilization and therefore many researches are being conducted to avoid these situations. These infections can be caused by road accidents, open wounds which are not treated in time and even by medical conditions such as poor sanitation of hospitals and surgical instruments used at the time of operation. Repeated use of antibiotics have failed to prevent infections as because the bacteria evolve themselves which make them resilient to medicines.The ECDE/EMEA(the European Centre for Disease Prevention and Control/the European Medicines Agency) has stated that the most deadliest of all antibiotic-resistant bacteria strains such as *Staphylococcus Aureus*MRSA(methicillin resistant) and *Staphylococcus Aureus* VISA/VRSA (vancomycin intermediate resistant, vancomycin resistant) are the cause of death of many patients[1]. Therefore finding new antibacterial agents is a promising approach to counter against the evolved bacterial trait. New approach such as using nanobiotechnology in making biomaterials is an effective way against bacteria. One of the most effective and promising materials are nanocomposites based on Silver(Ag) and Chitosan. The effectiveness of silver spins around its low propensity to choose for resistance, its wide range of uses and its chemotherapeutic ratio(toxic dose divided by effective dose). Besides Silver has the added benefit of being less toxic to human tissues than harmful microorganisms. Silver is toxic to microorganisms and has many ways to kill antibiotic resistant bacteria [2-10]. Because of the vicinity of functional groups (amino and hydroxyl), chitosan shows numerous fascinating properties [11]. Uses of chitosan are a result of its biodegradability, biocompatibility and non-toxicity. Additionally, chitosan shows an antibacterial movement against Gram-positive and Gram-negative bacterial strains [12]. Its action was exhibited at numerous microscopic organisms, growths and yeasts [13-14]. The antibacterial property of Silver and chitosan can be induced on 316L SS by suitable coating methods like sol gel, anodic oxidation, physical vapour deposition, electrophoretic deposition, electroplating and biochemical methods [15]. EPD is an effective way for coating Silver and chitosan as because of its simplicity, uniform coating and low deposition time. The charged particles which are to be deposited are driven by the electric field and gets deposited on the metallic substrate.

CHAPTER 2

LITERATURE REVIEW

2.1 Metallic biomaterials

The Metallic biomaterials have uique properties such as good biocompatible with the in-vivo enrironment of the human body, mechanical strength of the metallic biomaterials is comparable to human bones therefore reducing the risk of stress shielding and good load bearing capacity.The Corrosion resistance of the metallic biomaterials is better than other type of biomaterials. The ideal metallic biomaterials or implants show appropriate modulus of elasticity as the bone and exhibit imaging transparency. Currently, the metallic biomaterials like 316L Stainless Steel, Co-Cr alloys, Ti and its alloys are widely being used in orthopedics [16].

2.2 316L Stainless Steel

Implants and prosthetic devices are subjected to various stresses. These parts need to have sufficient mechanical strength to withstand the stresses. So the implants should exhibit good mechanical properties and load bearing capacities. These implants may also corrode inside the body when they come in contact with the body fluids, acids and enzymes. So they should have high corrosion resistance. They should have good fatigue life and appropriate Young's Modulus of elasticity. 316LSS exhibits all these properties and hence is a suitable biomaterial for load bearing applications[16].

Stainless steel contains 18 wt% Cr and 8 wt% Ni so that it becomes stronger and more corrosion resistant than the Steel. Addition of Molybdenum (Mo) to SS further improves its corrosion resistance and then it was called 316 Stainless Steel. If the carbon content in the 316 Stainless Steel is high, then the Cr present in 316 Stainless Steel will react with Carbon leading to the formation of a compound, Chromium Carbide. Then the iron surface in stainless steel will be exposed to oxygen and water and hence it will corrode. So the carbon content in stainless steel has been reduced from 0.08 wt% to 0.03 wt%. On account of low carbon content, 316 Stainless Steel was further named as 316L Stainless Steel. The presence of Chromium in Stainless Steel develops a passive oxide layer on the surface of the 316L Stainless Steel. This passive oxide layer acts as a protective film, which prevents the metal underneath from getting exposed to oxygen and moisture, thus increasing the corrosion resistance of the 316L Stainless Steel [16].

2.3 Bacterial infections (Microbiologically influenced corrosion) of 316LSS

Implant surfaces could be toxic in vivo if they undergo corrosion. Our body contains a large number of microorganisms that may grow on the implant surfaces and produce various metabolic by-products that may deteriorate the metallic implant surface. This phenomenonon known as bio corrosion. It is also referred to as Micro-biologically influenced corrosion (MIC) [17]. 316L Stainless Steel has been affected by various types

of corrosion, mainly the pitting corrosion that can be activated by the presence of microorganisms. These microorganisms have the ability to modify or mould the environment of the 316L Stainless Steel, thus creating crevices or cracks and zones of differential aeration with the help of metabolite formation[18].

2.4 Antibacterial metals development

Bacterial attachment, growth and proliferation on the material surfaces needs to be prevented and for that the antibacterial metals development becomes an area of concern[19].

According to a study, nine pure metals such as Ti, Co, Ni, Cu, Zn, Ag, Zr, Mo, SnandPb were tested of their antimicrobial properties against the two toxic bacterial strains of Gram positive Staphylococcus aureus and Gram negative E.coli. The results showed that different metals exhibited different antibacterial properties. While the metals like Zn, Ag and Cu exhibited high antibacterial properties, metals like titanium and tin did not at all exhibit antibacterial properties[19].

2.5 Silver as an antibacterial element

In last decade Silver has been used in various medical applications because of its good anti-bacterial properties such as wound dressing and urinary catheters.The amount of silver released from the coating determines the antibacterial activity of the Silver which if too high results in cytotoxicity.The use of the Silver in biomedical application has come into wide use for tropical treatment. The effectiveness of silver revolves around its low propensity to select for resistance, its broad spectrum of activity and its high chemotherapeutic ratio (toxic dose divided by effective dose). Silver is biocidal in the ionic form and, unlike many antibiotics, has at least six different mechanisms of action. Silver has the added benefit of being highly toxic to microorganisms with a relatively low toxicity to human tissues.Silver has been used for centuries to keep water clean and for other sanitary purposes, but recently it is receiving a lot of attention due to the rise in antibiotic resistant bacteria. Silver is a counter to this because it works via many ways, making bacteria l resistance very rare.

2.6 Polymers for coating the 316L Stainless Steel

2.6.1 Brief review of works on some polymeric coatings

It has been shown that Alginate-Hap composite scaffolds promote osteosarcomacell adhesion[20]. Potential bone tissue engineered alginate gel beads can be prepared by introducing the alginate gel beads into Calcium phosphate cement[21]. Gelatinis derived

from collagen through its partial hydrolysis. It is a mixturee of peptides and proteins. Gelatin promotes thrombogenicity and cellular proliferation[22]. Gelatin has high biocompatibility and better swelling ratio. So gelatin has been extensively used in drug delivery systems. Biocomposite scaffolds fabricated from Gelatin and bioactive nanoparticles of glass have porous three dimensional microstructures as observed under SEM. These scaffolds have density, porosity and elastic modulus very close to or in the range of natural bone[23]. So,gelatin promotes cell aggregation. In another work, a bioactive composite scaffold consisting of bioactive-glass and gelatin has been introduced through the method of direct foaming. This composite scaffold stands better than the polymer based scaffold as it allows direct bone tissue regeneration. So gelatin can be used to fabricate such composite scaffolds that can promote direct bone tissue regeneration.These scaffolds are biocompatible and non-degradable. They facilitated osteogenic differentiation and deposition of extracellular matrix[24].

2.6.2 Properties impartedby different polymeric or other coatings

- Polypyroleand Nb2O5 nanoparticles: Enhanced biocompatibility, enhanced corrosion resistance and mechanical strength.
- Hap nanoparticles: Improved corrosion resistance in the body, promotes implant fixation by chemical bonding, excellent load bearing capacities and enhanced bioactivity.
- Heparin: Cell adhesion,anti-coagulation and inhibition of intimal hyperplasia.
- Plasma polymerised Allylamine(PPAA): Improved cell adhesion and cohesion properties.
- Poly (PDMA: Corrosion inhibition and improved cell adhesion.
- 3-octyl-thiophene)-(P3OT) and Polystyrene (PS): Enhanced cell adhesion.

2.6.3 Chitosan

Chitosan exhibits biocompatibility, biodegradability and osteo-conduction properties. Chitosan coatings exhibit good biocompatibility, low degradation and processing ease and they also have the potential to swell and dehydrate depending on composition and environment. They promote cell growth and have good mechanical strength, high compression strength (7.68MPa) and elastic modulus of 0.46 GPa well matched with the bones. They facilitate cell spreading and proliferation. It has been shown that the development of a biodegradable porous scaffold made from chitosan and alginate polymers enhances the mechanical and biological properties of the coating, facilitates the attachment of the bone forming osteoblasts readily to the chitosan-alginate coating, well proliferation of osteoblasts and high degree of tissue compatibility[25].
In previous works, it has been shown that chitosan and chitosan mediated coatings facilitate the immobilization of proteins, nucleic acids and virus particles and it has also been proved that the surface properties of the implants, precisely metallic implants have been positively affected by the chitosan coating[26]. Development of a chitosan-HA coating on 316L Stainlesss Steel by EPD increases the corrosion resistance of the 316L Stainless Steel sample[27]. In a previous work, Chitosan-bioactive glass composite layer

was deposited on Titanium implants by EPD and the coated surface exhibited greater particle size, increased porosity and better corrosion resistance[28]. It has also been shown that deposition of a chitosan-titania nanoparticle composite coating on 316L Stainless Steel through EPD increases the corrosion resistance and hydrophilic tendency of the implant[29].

2.7 Electrophoretic deposition

Electrophoretic Deposition is a method by which the charged ions in the solution are attracted and gets deposited on the oppositely charged electrode under the influence of an electric field. First of all ,the charged ions move to the oppositely charged electrode under the influence of an electric field. Secondly, the particles that are stored on the electrode shape into a thick, compact film or layer that is further coated onto the metallic substrate.

CHAPTER 3

OBJECTIVE

OBJECTIVE

The work aims at the fabrication of a Silver-Chitosan composite coating on 316L Stainless steel by Electrophoretic deposition method., The following objectives are to be fulfilled.

1. To deposit Silver coating on 316L Stainless steel sample and a Silver-Chitosan coating on 316L Stainless steel by EPD method.

2. To find out the effective parameters (supply voltage and deposition time) for the deposition of Silver on 316L Stainless steel sample.

3. To study the effect of deposition time and supply voltage on the morphology of the coating surfaces.

4. To characterise the coated samples for their surface morphology analysis through optical microscopy and FESEM and phase purity analysis and crystallinity through XRD.

5. To check the antibacterial property due to the presence of Silver and Chitosan coating on the 316L Stainless steel.

CHAPTER 4

MATERIALS AND METHODS

4. MATERIALS AND METHODS

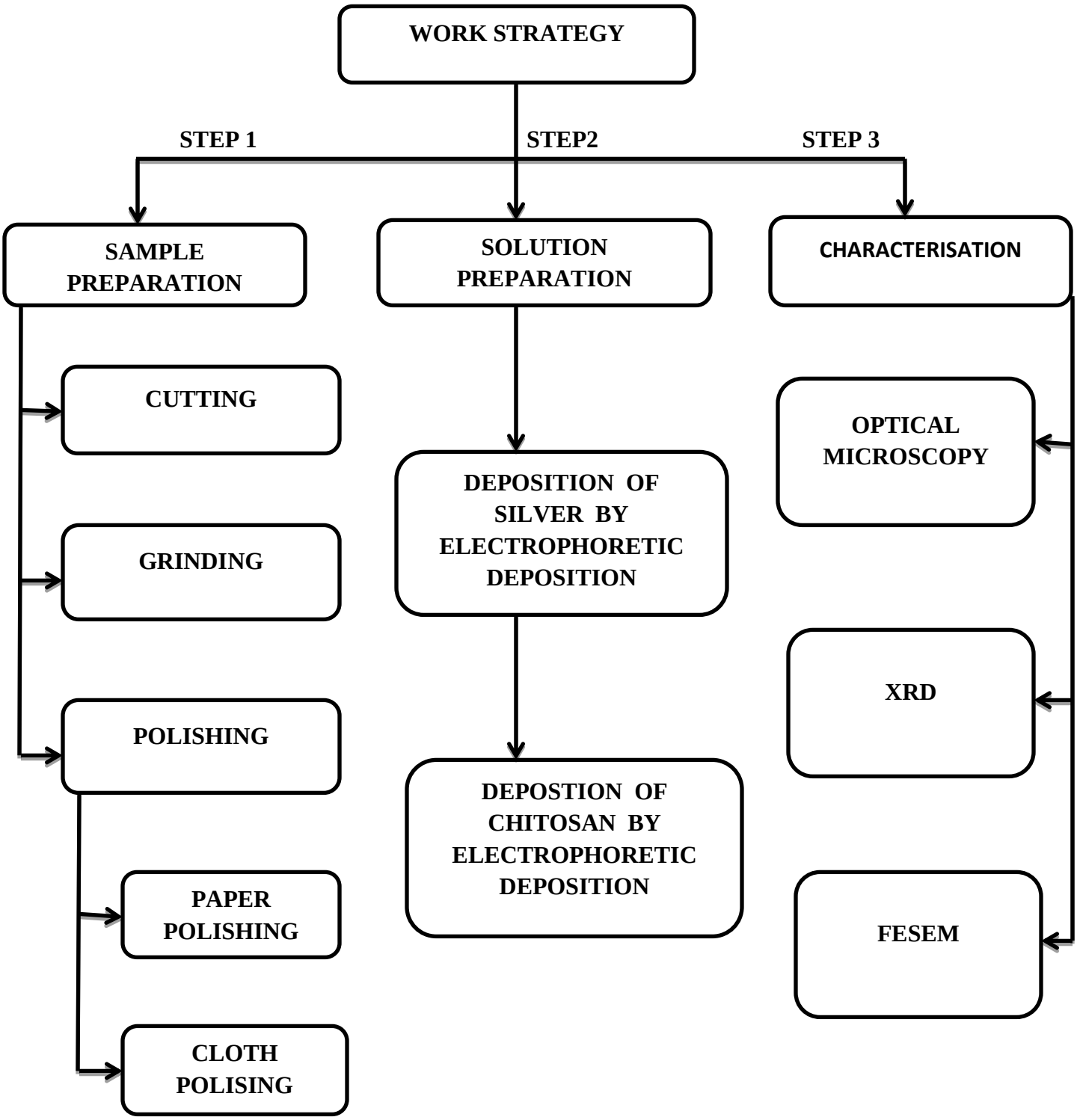

Figure 1: Schematic representation of the work strategy

REQUIREMENTS:-

1) . For Sample preparation

I. 316L Stainless Steel sheet
II. Grinder
III. Emery polishing paper(1/0,2/0,3/0)
IV. Distilled water for sample cleaning

2). For coating Silver and chitosan by electrophoretic method

I. A discharged dry cell pencil battery
II. Silver Nitrate powder
III. Chitosan powder
IV. Acetic acid
V. Magnetic Stirrer
VI. Muffle Furnace
VII. Controlled DC supply

3). For characterisation of the prepared sample

I. Optical Microscope
II. X-ray Diffractometer
III. Nova Nanosem FESEM machine

PROCEDURE:-

4.1 Sample Preparation

a) Cutting the 316L stainless steel sheet:- A 316L Stainless steel sheet was cut into rectangular shapes with dimensions 2.5 cm x 1.5 cm. These were the samples on which silver and chitosan was performed.

b) Grinding:-These samples were then grinded in a belt grinder to remove the corroded layers and finishingthe surface.

c) Paper polishing:- Emery polishing papers were used to perform the paper polishing of the samples. The polishing was done in various grades of emery polishing papers in a sequential manner. First it was done on 1/0 grade emery paper, then on 2/0 grade emery paper and lastly on 3/0 grade emery paper. In each and every grade of emery paper, longitudinal and transversal orientation of the samples was performed alternatively .Finally a mirrored polished surface was obtained after the paper polishing was done on the samples. A mirrored surface is necessary for efficient coating of silver and chitosan.

d) Cloth polishing:-The samples were then cloth polished on a rotating disk covered with a fine cloth. Lubricants like alumina paste was poured on the rotating disk to enhance a good polished surface.

e) The polished samples were cleaned with DI water, dried and kept in a air tight container so as toprevent corrosion.

4.2 Coating of silver on the samples:-

a) Graphite electrode was taken out from a discharged dry cell pencil battery by removing the outer covering of the battery. The inner core contains the graphite electrode which was extracted out of the battery after the black coloured powder was removed. This electrode was cleaned by using DI water and ethanol. Precaution to use gloves for removing the graphite electrode should be taken as it is harmful for the skin.

b) Preparation of the electrolyte medium:- 0.5M $AgNO_3$ electrolytic solution was prepared by dissolving 1.698 gram silver nitrate solute in 20ml of distilled water. The solution was mixed homogenously with the help of a magnetic stirrer and a magnetic bead. The rotating speed of the stirrer was set to 300 rpm at room temperature.

c) Electrophoretic deposition of silver on 316L stainless steel sample:- The graphite electrode was used as an anode and the 316L stainless steel sample was used as the cathode. Both these electrodes was dipped in the $AgNO_3$ electrolyte solution

in a small beaker and a constant DC supply was applied for every sample. The process parameters for silver deposition on 316L stainless steel are:-

Supply Voltage	Deposition Time		
3.7V	5 min (Sample 1)	3 min (Sample 2)	1 min (Sample 3)
2.7V	5 min (Sample 4)	3 min (Sample 5)	1 min (Sample 6)

Table 1: Process parameters for Silver Deposition

The following care should be taken while Silver coating is deposited on 316L stainless steel:-

a) The anode and the cathode should not touch each other .
b) The electrodes should not touch the glass of the beaker.
c) The iron clamps should not dip in the electrolyte.
d) The electrodes should be 2cm apart from each other.
e) Both the electrodes should be dipped at an equal level.

The coated samples were then placed in the muffled furnace at 500°C for 5 mins as because the silver was adhered loosely to the 316L Stainless steel. So post heating treatment should be done for silver to adhere tightly to the 316L Stainless steel sample.

4.3 Coating of Chitosan on Silver-coated 316L stainless steel:-

a) Preparation of Chitosan solution: 0.8 gram chitosan was dissolved in 500ml of acetic acid + water. Acetic acid is added to water because chitosan does not dissolve in distilled water and acetic acid helps in dissolving the chitosan. The solution was kept in a magnetic stirrer using a magnetic bead in the solution. The solution was kept overnight at a rotating speed of 400rpm.
b) 150ml of the solution was taken in a beaker and electrophoretic deposition was carried out.
c) The graphite rod was used as the anode and the Silver-coated 316L stainless steel sample was used as the cathode.
d) The EPD was performed at a constant DC voltage of 20V and the deposition time of 15min.

Solvent used	Acetic acid- 20ml Water-480ml
Solute used	Chitosan
PH	3.3-3.6
Applied Voltage	20V
Deposition Time	15 min

Table 2:Process parameters for Chitosan Deposition

4.4 Characterization Techniques:-

Characterization techniques such as surface morphology analysis like Optical microscopy and FESEM was used to analyze the surface morphology of the prepared samples. Phase purity analysis was done by XRD technique.

Surface morphology analysis:

4.4.1 Optical Microscopy-An inverted metallurgical optical microscope manufactured by ChennaiMetCo with an image analyzer software was used for surface morphology analysis of the silver-coated 316L Stainless steel. The prepared sample was placed over the horizontal stage with surface perpendicular to the optical axis of the optical microscope. Light from a source was used to illuminate the sample through the objective lens. This light was then focussed by a condenser lens into a beam, whose orientation was adjusted parallel to the optical axis of the optical microscope with the help of a half silvered mirror.

4.4.2 Phase purity analysis

The phase identification and crystallinity of the coated material was analysed by the XRD that is a technique used to characterise the compounds based on the diffraction pattern of the constituent elements. The X-ray diffractometer, and the data collector software were used to perform the XRD characterisation of the sample. The XRD profile was obtained in the range of 5 to 95 and the scan rate was maintained at 3°/min. The XRD profiles of the Silver coated 316L Stainless Steel sample and Silver–Chitosan coated 316L Stainless Steel sample thus obtained were compared with the standard JCPDS data of Silver and Chitosan. The peaks obtained in the XRD profile and the corresponding angles were studied and compared with the standard JCPDS so as to identify the components present in the coatings.

4.4.3 FESEM

The threeSilver – Chitosan coated 316L Stainless steel sample were characterised using FESEM technique. The surface needed to be made conductive for performing FESEM. The deposition of Chitosan on the Silver coated 316L Stainless steel sample made its surface nonconductive. To make the surface conductive, Pt sputtering was performed on the non-conductive Chitosan coated 316L Stainless Steel surface. By FESEM the data about the surface morphology of the coated sample was acquired.

4.4.4 Antibacterial effect test

a) Nutrient Agar medium(2%) was used for culturing *Escherichia Coli.*
b) The media was prepared by adding 1.8 gram of Nutrient agar powder in 100ml of water. The solution was mixed properly and was autoclaved for 20minutes.
c) At laminar flow condition,30ml of the prepared media was poured into a petriplate before it solidified.
d) 1ml of E.Coli was dropped in the petriplate and spread evenly with the help of rod whose tip was covered with wool.
e) A 316L Stainless steel sample, a Silver-coated 316L Stainless steel sample and a Silver-Chitosan coated stainless steel sample were placed in the petriplate at equal distances from each other. The 316L stainless steel sample was used as a positive control for the testing.
f) The petriplate was wrapped tightly with parafilm and was kept in the incubator at 37 degree Celcius overnight.
g) The next day, the petriplate was observed for any “Zone of Inhibition”.

CHAPTER 5

RESULTS AND DISCUSSION

5. RESULTS AND DISCUSSION

This section briefly describes the outcome of the electrophoretic deposition of Silver and chitosan composite on 316L stainless steel and how it changed the property of the 316L stainless steel. The coated samples were checked for the change the surface morphology, and checked whether the silver-chitosan was effectively deposited on 316L stainless steel. The results obtained from the different characterization techniques are discussed below.

5.1Optical microscopy

Figure 5(a), 5(b) and 5(c) below show the optical microscopy images of silver coated 316L Stainless steel samples. Figure 5(a) is the sample in which Silver was deposited at 3.7V for 3mins. Figure 5(b) is the sample in which Silver was deposited at 2.7V for 5mins. Figure 5(c) is the sample in which Silver was deposited at 2.7V for 1min. These 3 samples were best suited for Chitosan coating as Silver was deposited in minute amounts on the 316L Stainless steel resulting in a non-uniform deposition and very minute amount of Silver can exhibit antibacterial effects. With increase in the supply voltage keeping the time duration constant, Silver deposition rate increases. With increase in the time duration keeping the supply voltage constant, Silver deposits uniformly in high amount over the surface of 316L Stainless steel.

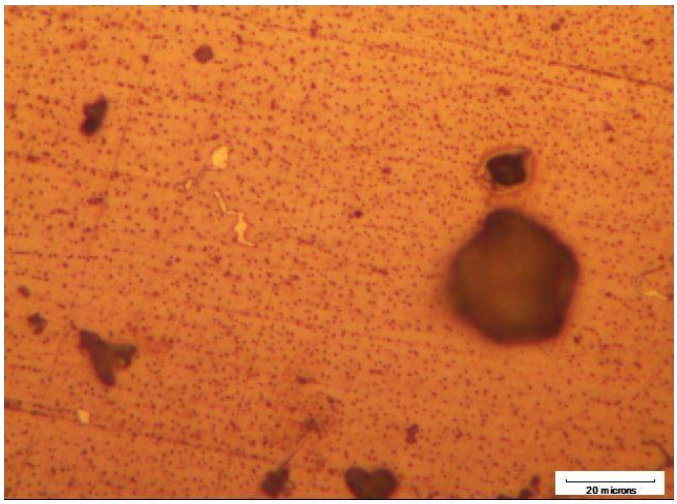

Figure 5(a) Optical microscopy of Ag coated 316L SS(3.7V,3 min)

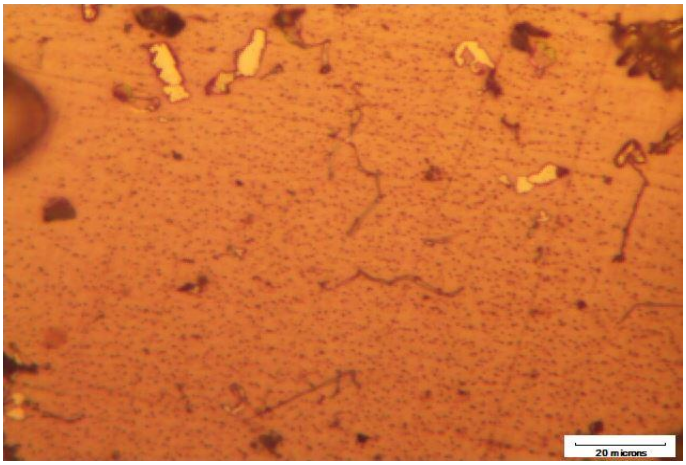

Figure 5(b) Optical microscopy of Ag coated 316L SS(2.7V,5 min)

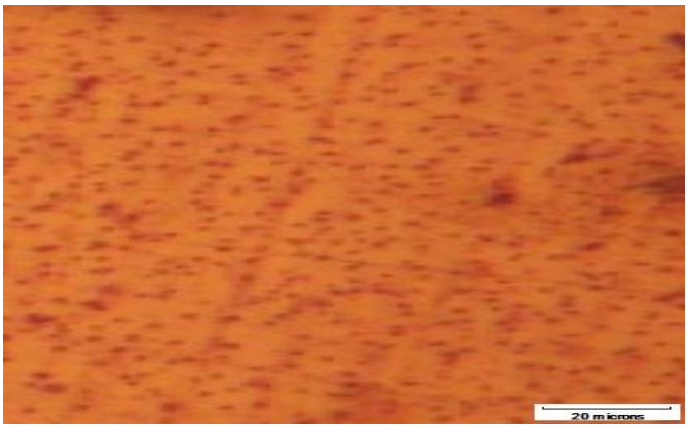

Figure 5(c) Optical microscopy of Ag coated 316L SS(2.7V,1 min)

5.2 XRD analysis

The XRD profile of Silver coated 316L stainless steel sample with deposition supply voltage of 3.7V and deposition time of 3 minutes was generated. Further this coated sample on which deposition of chitosan was carried out at supply voltage of 20V for 15 minutes was characterized by XRD and the XRD profile was generated.

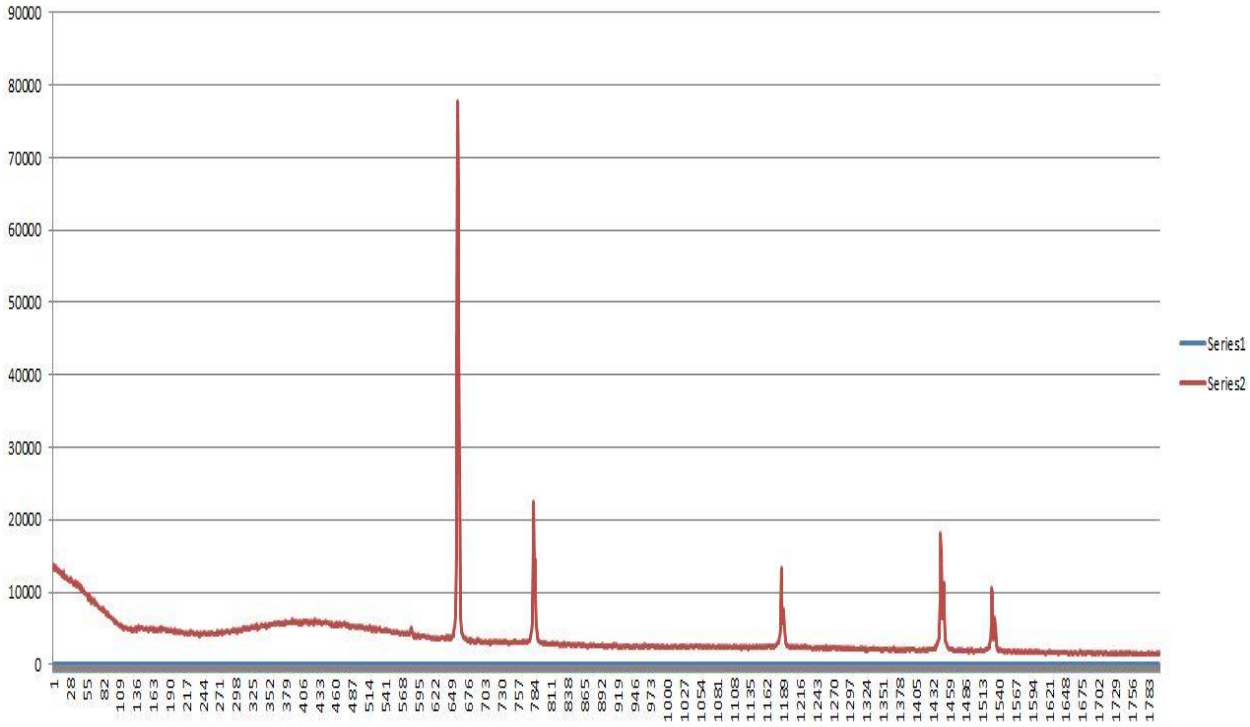

Figure 6(a):XRD profile of silver coated 316L stainless steel

Peak list of Silver coated 316L Stainless steel

Peak list

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM(deg)	Int. I(cps deg)	Int. W(deg)	Asym. factor
1	34.064(5)	2.6298(4)	1099(61)	0.09(4)	119(27)	0.11(3)	0.3(6)
2	37.884(2)	2.37295(13)	70899(486)	0.108(3)	9780(141)	0.138(3)	0.56(4)
3	44.077(2)	2.05284(11)	20050(259)	0.090(3)	2896(30)	0.144(3)	0.69(9)
4	64.246(5)	1.44860(10)	10573(188)	0.102(4)	1712(24)	0.162(5)	1.0(2)
5	77.220(4)	1.23440(5)	17336(240)	0.109(4)	2979(35)	0.172(4)	1.11(18)
6	81.374(5)	1.18155(6)	9812(181)	0.104(8)	1557(25)	0.159(5)	1.2(3)

Table 3:Peak list ofSilver Deposition on 316L SS

Measurement profile

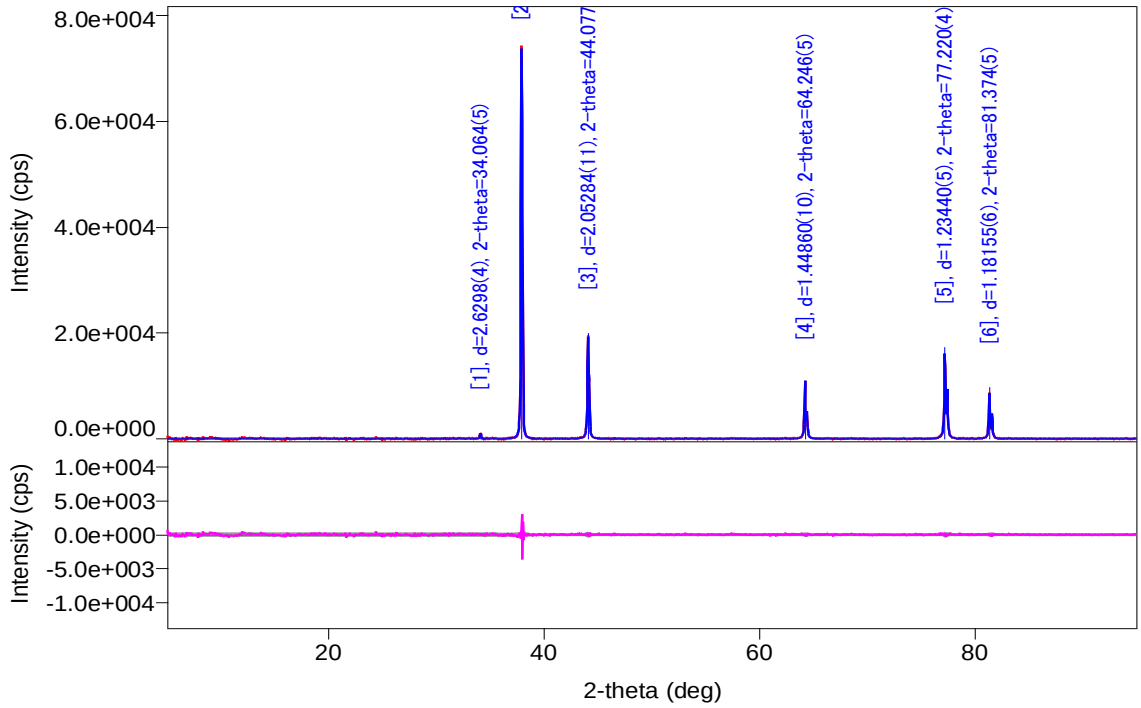

Figure 6(b) Peaks of Ag coated 316L SS

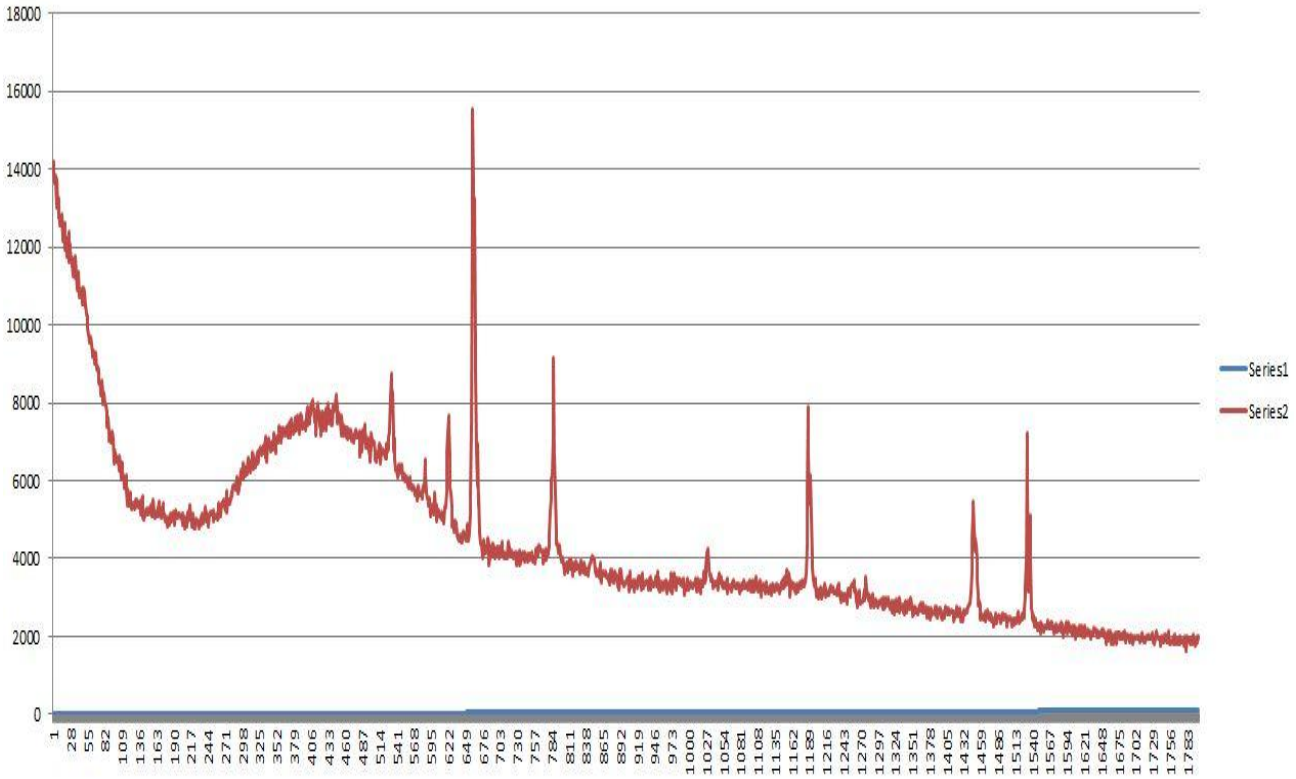

Figure 6(c):XRD profile of Ag-Chitosan on 316L SS

Peak list of Silver-Chitosan coated on 316L SS

Peak list

No.	2-theta(deg)	d(ang.)	Height(cps)	FWHM(deg)	Int. I(cps deg)	Int. W(deg)	Asym. factor
1	31.563(14)	2.8322(12)	2033(82)	0.15(3)	605(30)	0.30(3)	1.8(9)
2	34.20(3)	2.619(2)	800(52)	0.11(3)	156(21)	0.19(4)	1.2(10)
3	36.049(6)	2.4894(4)	2136(84)	0.20(2)	574(33)	0.27(3)	1.6(15)
4	37.937(13)	2.3698(8)	10289(185)	0.14(2)	2635(127)	0.256(17)	0.7(3)
5	44.300(5)	2.0430(2)	4121(117)	0.114(12)	958(39)	0.232(16)	2.0(13)
6	56.42(3)	1.6296(7)	805(52)	0.18(3)	180(22)	0.22(4)	2.2(18)
7	64.313(8)	1.44727(17)	4693(125)	0.124(9)	906(24)	0.193(10)	1.0(3)
8	77.281(10)	1.23357(14)	2856(98)	0.198(11)	844(24)	0.295(19)	1.2(3)
9	81.517(18)	1.1798(2)	5735(138)	0.08(3)	769(45)	0.134(11)	1.1(10)

Table 4:Peaks list for Silver-Chitosan Deposition on 316L SS

Measurement profile

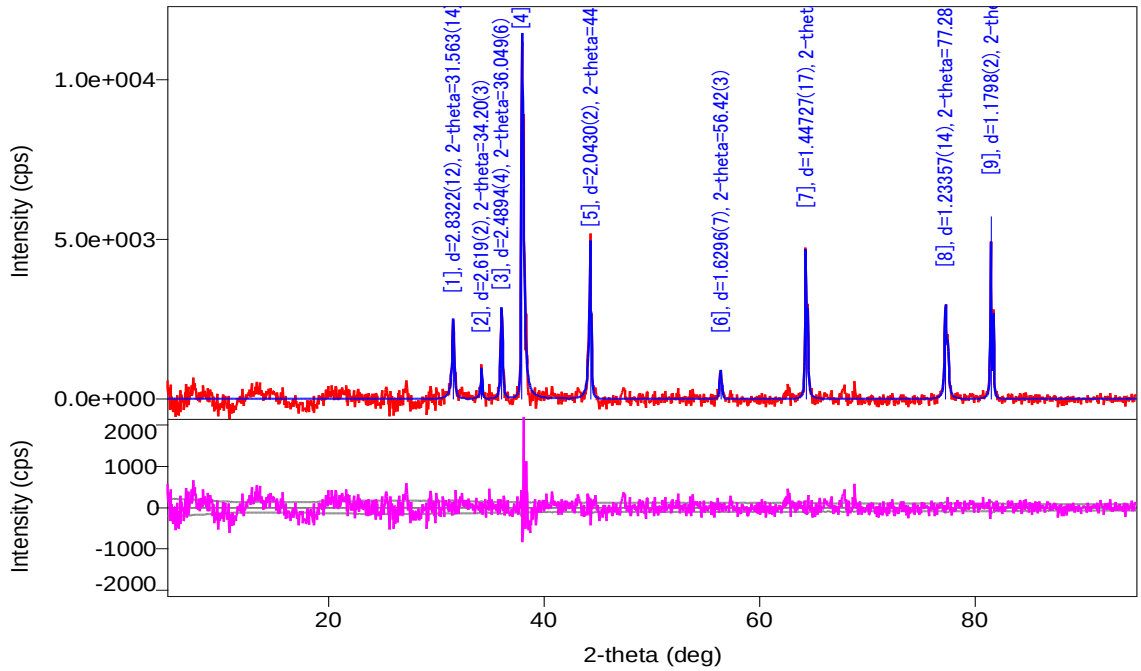

Figure 6(d):Peaks of Ag-chitosan on 316L SS

Figure 6(a) shows the XRD profile of Ag coated 316L Stainless steel. High intensity peaks were observed at 2 theta values of 34.064 degree,37.884 degree,44.077 degree,64.246 degree,77.220 degree and 81.374 degree. According to JCPDS of Silver, the XRD peaks of Silver is obtained at 2 theta values of 38.3182 degree, 44.4975 degree, 64.6119 degree, 77.5385 degree and 81.6839 degree. Thus it is confirmed that Silver has been successfully deposited on the surface of 316L Stainless steel. The intensity of the peaks depicted that the silver nanoparticles are crystalline in nature. But the diffraction peak are broad which shows that crystallite structure of silver nanoparticles are very small.

Figure 6(b) shows the XRD profile of Silver-chitosan coated on 316L stainless steel. High intensity peaks were observed at 2 theta values of 31.563 degree, 34.20 degree,36.049 degree, 37.937 degree, 44.300 degree, 56.42 degree, 64.313 degree, 77.281 degree and 81.517 degree. All these peaks correspond to the JCPDS data of Silver. Small intensity peaks were observed at 2 theta values of 24.45 degree and 27.073 degree. These small peaks corresponds to the JCPDS data of chitosan. This concludes that Silver and chitosan were successfully deposited on the surface of 316L Stainless steel.

5.3 FESEM analysis for surface morphology

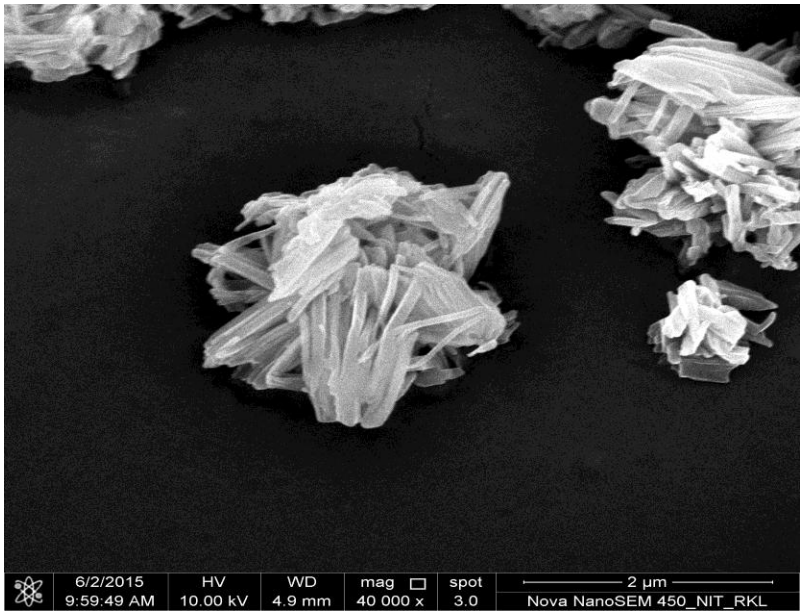

Figure7(a):FESEM of Ag-chitosan(Ag at 3.7V for 3min,chitosan at 20V for 15 min-40000X)

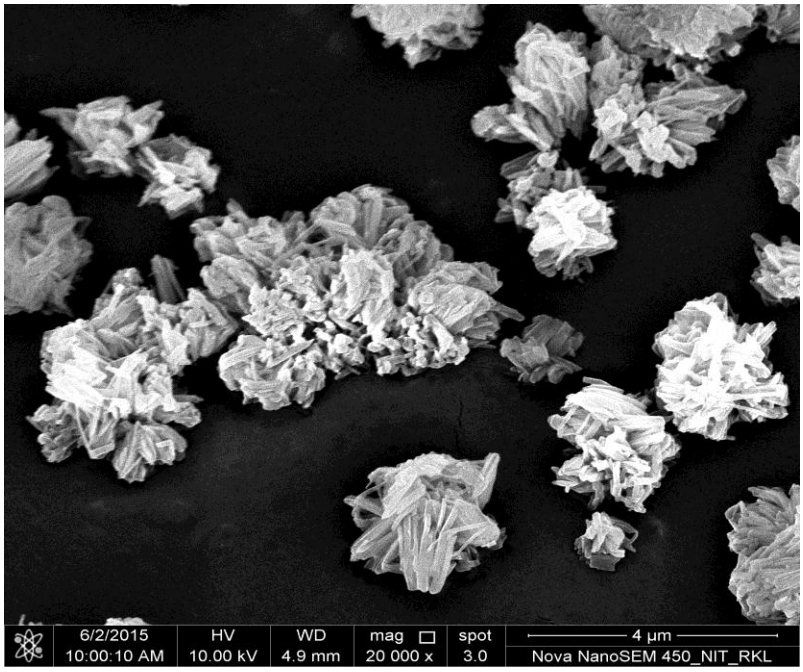

Figure7(b):FESEM of Ag-chitosan(Ag at 3.7V for 3min,chitosan at 20V for 15 min-20000X)

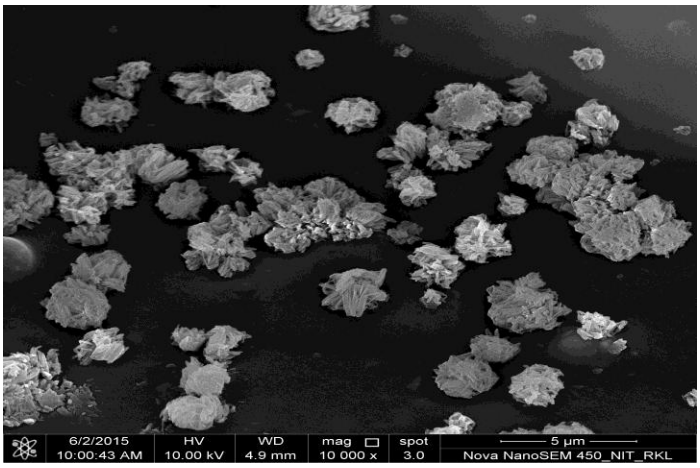

Figure 7(c)FESEM of Ag-chitosan(Ag at 3.7V for 3min,chitosan at 20V for 15 min-10000X)

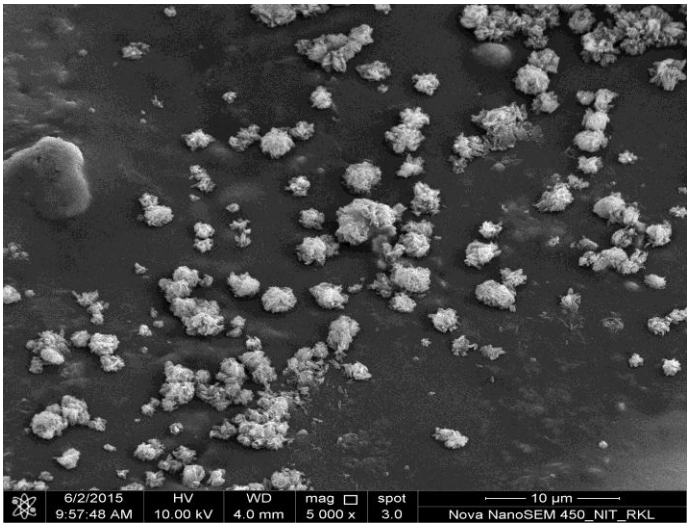

Figure 7(d)FESEM of Ag-chitosan(Ag at 2.7V for 5min,chitosan at 20V for 15 min-5000X)

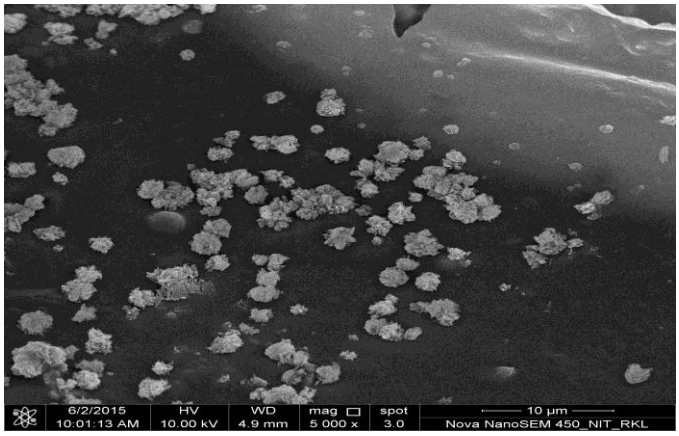

Figure 7(e)FESEM of Ag-chitosan(Ag at 2.7V for 1min,chitosan at 20V for 15 min-5000X)

Figure 7(a),7(b),7(c),7(d) and 7(e) above shows Ag-Chitosan deposition at 20V supply voltage and deposition time of 15 min and was carried out on the Silver coated sample carried out at 3.7V(3 minutes), 2.7V(5 minutes) and 2.7V(1 minute) by EPD method. This Silver-chitosan coated sample under FESEM showed fine grains structure confirming presence of both Silver and Chitosan on the 316L stainless steel. The Silver and chitosan forms composite and gets deposited on the surface of 316L Stainless steel.

5.4 Antibacterial effect testing results after 36 hours of incubation

The polished 316L Stainless steel sample does not clear out the bacterial zone, but the area around the Ag coated 316L stainless steel and Ag-chitosan coated 316L stainless steel showed zone of inhibition as no bacteria were found in that zone. This confirms the antibacterial property of both silver and chitosan and these can be utilised to modify the surface of biomaterials to make it antibacterial.

CONCLUSION

CONCLUSION

After following all the work strategy and methods and analysing the results, we can conclude that the Silver and chitosan were successfully deposited on the 316L Stainless steel.

• The surface of 316L stainless steel was modified to become antibacterial surface by the deposition of silver and chitosan .An effectiveAg-Chitosan coating was obtained at Ag deposition parameters of 3.7V(3 minutes), 2.7V(5 minutes),2.7V(1 minute) and on this sample Chitosan was deposited at supply voltage of 20V and deposition time of 15 min.

• The variation of deposition time and the DC supply voltage produced different amount of coating on 316L stainless steel as studied by Optical microscopy and FESEM analysis.

• Silver and Chitosan was confirmed on the surface of the samples bythe XRD analysis.

• The antibacterial property of the Silver and Silver-Chitosan was affirmed by checking the zonal clearance of the area of microbes in the petri dish close to the region of the Silver covered 316LSS and Silver-Chitosan coated 316L SS.

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