# Hydroxyapatite Coating on Selective Laser Sinter Polyamide Substrate by Electron Beam Deposition

HARIHARAN K[1*] AND ARUMAIKKANNU G [2]

*[1]Department of Mechanical Engineering, Dr. Mahalingam College of Engineering and Technology, Pollachi- 642003, India*
*[2]Department of Manufacturing Engineering, College of Engineering Guindy, Anna University, Chennai - 600025, India*

**ABSTRACT**

*Polyamides 12 (PA-12) has an application in the medical field such as suture materials, scaffolds for tissue engineering and for various medical devices fabrication. More often the medical devices which are produced using PA-12 are addressed to be a bioinert and osseointegration with the host tissue will be in the slower rate. This experimental work aims to improve osseointegration of PA-12 by depositing a layer of bioactive material over the surface, since the surface is the first portion to interact with host tissue. Hydroxyapatite (HA) is a calcium phosphate similar to the human tissue in morphology and composition also it is well known bioactive material. This material has been synthesised by wet chemical precipitation process and deposited over polyamide substrate using Electron Beam Deposition (EBD). Various characterization studies have been carried out on coating and Human Osteosarcoma cells MG-63 were seeded on the coated sample to study its viability. The results revealed that Hydroxyapatite layer was deposited successfully over the PA-12. The coated layer some agglomeration of particles. The cell possesses 92.3% of viability, which may significantly improve the biocompatibility of prostheses and medical devices.*

KEYWORDS *: Polyamide, Hydroxyapatite, Wet Chemical Precipitation, Electron Beam Deposition, Characterization, Invitro analysis*

## 1. INTRODUCTION

Implants made with metals and metallic alloys were popular in replacing the bone in terms of plates, screws and pins; artificial joints, temporary and permanent fixation but due to their high modulus, high yields points and

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Correspondence author e-mail: khariharan247@gmail.com

ductility compared to natural bone, the fatigue failure due local stress induced on the implant in the elastic range [1], the wear followed by mechanical damage results in premature removal of the implants, the micro debris generated by wear will lead to loosen the implants and the alloys [2]. The presence of alloying elements such as Ni, Cr, and Co in both stainless steel and Co-Cr alloys has toxic effects, Ni toxicity leads to dermatitis. The long-term existence of Al and V ions in Ti alloys has been found to cause Alzheimer's disease, osteomalacia, and neuropathy in the long term. The presence of Co has also been reported to have carcinogenic effects [3].

For the past few decades, increases in the number of polymers were in large scale also it has been examined for biomedical uses, since polymer consists of carbon atom backbone (which determines a property know as tacticity) it is suitable for medical usage rather than inorganic material [4]. Also polymers mostly address the shortcomings of metallic biomaterials in terms of mechanical properties, corrosion resistance and chemical nature. Among different polymers available, polyamide was one of the preferred candidates to produce the implants, dental screws, sutures, tendons replacements and cancellous bone replacements[5]. From various polyamides, PA-12 will offers an excellent balance of properties and it is very popular material which holds a wide range of application both in industrial and medical [6]. PA-12 can be fabricated porous or softer form and can be manipulated easily and allow better reproduction. More importantly as other polymer, PA-12 does not generate electrolytic current prone to corrode and shows fibrous connective tissue attachment. This material provide suitable alternatives to bone implants while also providing the capability to be custom manufactured with respect to patient specific data and targeted application [7-9].

Custom made implants offer potential for improving the way how bone defects are repaired [10]. Such implants would be designed and optimized for a target defect using anatomical information, functional design criteria and surgeon input. The implant would be made from suitable material with desired properties and implanted in the patient to affect repair and restore shape; and functions with the help of measurement data such as tactile, optical or medical imaging methods such as CT or MRI, custom implants are designed with computer aided CAD software [11]. The combination of 3D implant model and Additive Manufacturing (AM) technologies offers the opportunity to produce anatomically tailored implants from variety of biomaterials including polyamides [12]. AM has ability to form strong, complex implants would alleviate existing problems with current materials and would open a new realm in the fabrication and treatment of bone defects. Selective Laser Sintering (SLS) is one of the AM technologies capable of fabricating complex anatomical structure directly from variety of materials including metals, polymers and ceramics [13].

The selection of proper biomaterials and fabrication of custom implants alone doesn't contribute to the success of implantation. The osseointegration is the key property which decides the long term stability of any implants [14, 15]. Even though PA-12 contains same amide

linkage as found in polypeptides their rate of degradation is low that often it is reported as non-degradable which also means that PA-12 has slow osseointegration. To improve the osseointegration of PA-12, bioactive materials can be physically blended with polyamide or coated over the surface of scaffold or implant [16]. Since the surface of the implant is the first part which interacts with the host tissue depositing a layer over the surface has a good impact on improving osseointegration [17]. Surface coating technology has attracted considerable attention of the researchers to develop novel coatings with enhanced functional properties like hardness, biocompatibility, wear and corrosion resistance for implants, medical devices and surgical tools, bioactive materials like hydroxyapatite, zirconia, and alumina were commonly deposited over the implants [18]. Hydroxyapatite (HA) powder was a frequently used bioactive material, since its chemical composition and properties are very similar to the human bone. The HA powder coatings on implant are widely used in orthopaedic applications and quantitative understanding of HA powder coating deformation might enable us to elucidate the mechanical stability of such implants in clinical applications [19]. Laboratory research also supports the conclusion that the early bone growth is accelerated by implants coated with HA powder. Hence the main objective of this work is set to enhance the osseointegration of PA-12 by depositing HA using EBD. Surface microstructure, crystal structure, adhesion strength of coating and the cell viability were investigated to quantify the HA coating, which will favour the biocompatibility by bone-implant anchoring.

## 2. METHODS AND MATERIALS

### 2.1 Fabrication of Laser Sintered PA 12 Substrate

The schematic diagram of SLS is shown in the Figure 1. As like other AM machines, this consists of three chambers, the extreme two chambers are known as powder delivery chamber and middle one is known as build platform. A PA 12 substrate (glass slit like structure) fabricated by SLS machine starts as a CAD file with dimension of 25×25×3 mm, then this file is converted to .STL format, which can be understood by

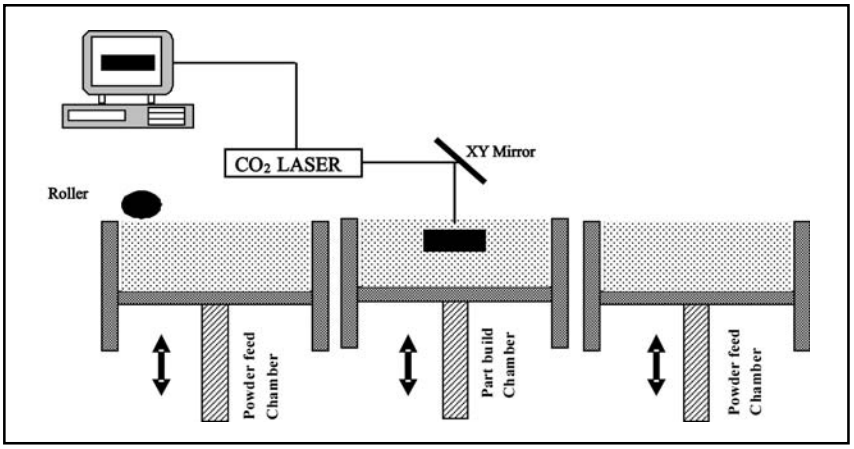


Fig. 1. Schematic diagram of SLS

an AM machine where it is sliced to number of layers (0.1 mm layer thickness). The sliced data was then loaded to eos Formiga P110 machine. A high power $CO_2$ (70 W) laser fuses fine PA 12 powder in 0.1 mm layers, navigated by a computer guided mirror. The build platform progresses downwards in steps of layer thickness. The delivery chamber provided with roller alternately rise and supply a fresh powder to spread accurately over the surface of the previously build area. The non-sintered powder particle forms "softy cake" like structure which enfold and supports the model as the build progresses. The entire process take place in a nitrogen environment to avoid the oxidizing the polyamide, when heated by the laser beam. The build chamber temperature is maintained at 170 °C, just below the melting point of polyamide material, so that once the laser comes into contact with the particles on the surface, they immediately fuse with the temperature rise of 12 °C. The process repeats itself over and over again until the entire object has been fabricated. Once the entire process is completed, the part is removed from the chamber and post processing like removal of un-sintered powder, clean-up and finally blasting with fine abrasive powder to have a proper surface [20, 21].

### 2.2 Synthesis of Hydroxyapatite Target

Hydroxyapatite (HA) powder used for deposition was synthesised by wet chemical precipitation process. A suspension of 0.5 M of Calcium Hydroxide $Ca(OH)_2$ in 1000 ml of distilled water was stirred vigorously for 30 min in magnetic stirrer and 0.3 M of Ammonium Phosphate $(NH_4)_2HPO_4$ in 1000 ml distilled water was stirred vigorously for 15 min and added drop by drop to the previously prepared $Ca(OH)_2$ solution. During the synthesis, pH was maintained at 7 and above; liquid ammonia was used to maintain the pH level. After 1 to 2 hours of preparation, a gelatinous precipitate was obtained and it was calcined at a temperature of 100°C for 5 hours to obtain HA powder. The prepared HA powder was made into target pellets with high pressure hydraulic press with 400 MPa load and sintered at 800°C for 5 hours [22].

### 2.3 Electron Beam Deposition of Hydroxyapatite

The laser sintered PA 12 substrate was ultrasonically cleaned using acetone and ethyl alcohol. For deposition, electron beam deposition (HIND HIGH VACCUM, INDIA) was employed. Electron beam deposition is a type of physical vapour deposition in which a target anode is bombarded with an electron beam delivered by a charged tungsten filament in an ultra high vacuum condition [23]. The electron beam causes atoms of the target to be transform into the gaseous phase and then these atom forms as a precipitate on the substrate which is fixed perpendicular to the HA target. The deposition chamber was evacuated to a base pressure of $5x10^{-4}$ torr. Before switching the electron beam, the deposition chamber is cleaned with $Ar^+$ ion beam, electron beam can be generated by thermionic emission and beam generated is accelerated to a high kinetic energy and directed towards the HA target. After striking on the HA target the electron lose their energy very quickly. The kinetic energy is converted to thermal energy through interaction with HA target. The thermal energy produced over the surface will cause the material to melt or sublimate. Once the vacuum level and temperature are in sufficient level, gaseous phase or vapour will be formed from the melt target material. The resultant vapour then passes on the chamber and deposited over the polyamide 12 substrate which is rotating at a speed of 10 rpm to obtain uniform deposition. During deposition, the high vacuum was attained at the range of $10^{-7}$ to $10^{-6}$ torr and e-beam was generated at operating voltage of 7 kV to vaporise the HA target [24].

### 2.4 *in vitro* Analysis

*in vitro* studies are performed with microorganism cells in a controlled environment outside of a living organism. Human Osteosarcoma MG-63 cell has been used for the *in vitro* cell viability analysis. This kind of cells is suitable for screening large number of samples for cytotoxic compound and also used in the rapid evaluation of the biomaterial surface qualities.

#### 2.4.1 Cell Culturing

The cellular response to the coatings was assessed in terms of cell viability and proliferation to the surface. Cells used for seeding were grown in 75 $cm^2$ flask containing Dulbecco's modified Eagle's medium (DMEM; Sigma). The mediums were supplemented with 10% Fetal bovine Serum (FBS; invitrogen), 1.5 g/l sodium bicarbonate, 10,000 Units/ml penicillin, 10 mg/ml streptomycin and 25 µg/ml Ampotericin B. Cells were

cultured as monolayers in culture flasks at 37°C under a humidified atmosphere of 5% $CO_2$ in air.

#### 2.4.2 Cell Seeding on Hydroxyapatite Coated Substrate

The coated substrate was sterilized with 70% ethanol for 30 min and then further it was autoclaved for 30 min, followed by drying at room temperature for 2 hrs. The cells were seeded approximately $1\times10^5$ cells/ sample. The cell proliferation and viability was assessed and observed at specific time period of 1st, 7th, and 15th day respectively.

### 2.5 Cell Viability

The viability of cells was assessed by standard MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [25]. This assay is based on the reduction of soluble yellow tetrazolium salt to insoluble purple formazan crystals by metabolically active cells. Only live cells are able to take up the tetrazolium salt. The enzyme (mitochondrial dehydrogenase) present in the mitochondria of the live cells is able to convert internalized tetrazolium salt to formazan crystals, which are purple in color. Then the cells were dissolved in DMSO solution. The color developed is then determined in an ELISA reader at 570 nm of UV-absorbance wavelength.

## 3. RESULTS AND DISCUSSION

### 3.1 Surface Morphology Analysis

The surface of hydroxyapatite coating was characterized using SEM; Figure 2 (a) shows that a dense and uniform layer was formed over the surface, further the examination revealed that the surface of the coating was free from defects like cracks, pores and large voids. Some isolated particles were identified on the layer from Figure 2 (b), this may be due to agglomeration of smaller particles. This kind of particle agglomerations will increase the surface roughness and help in improving osseointegration. Figure 2 (c) shows the cross-sectional image of HA layer with a thickness of 200 nm. The average surface roughness ($R_a$) of deposited layer was 3.41 µm measured using stylus probe technique. It has been demonstrated that, rough surface will stimulate better osseointegration [26, 27].

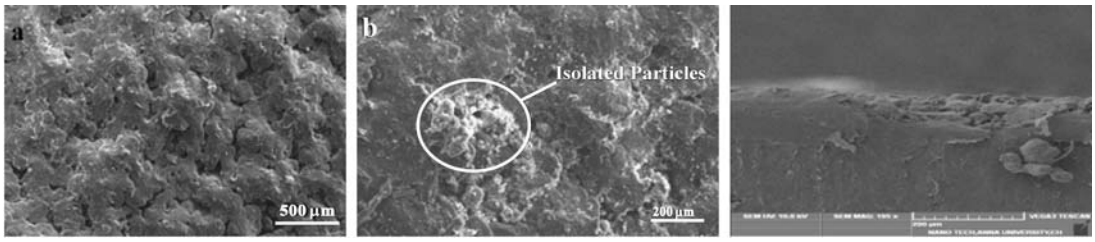


Figure 2 SEM Micrograph (a) Surface (b) Isolated particle (c) Cross sectional image

### 3.2 Elemental Analysis

The Elemental composition of a coated layer was analysed using EDX and the spectrum was shown in Figure 3. It shows that the characteristic peaks of calcium, phosphate and oxygen elements confirming that the layer was hydroxyapatite. These elements are main constitutes of hard tissue, teeth and tendons. The ratio of calcium and phosphate was approximately 1.68, which is close to that of standard HA.

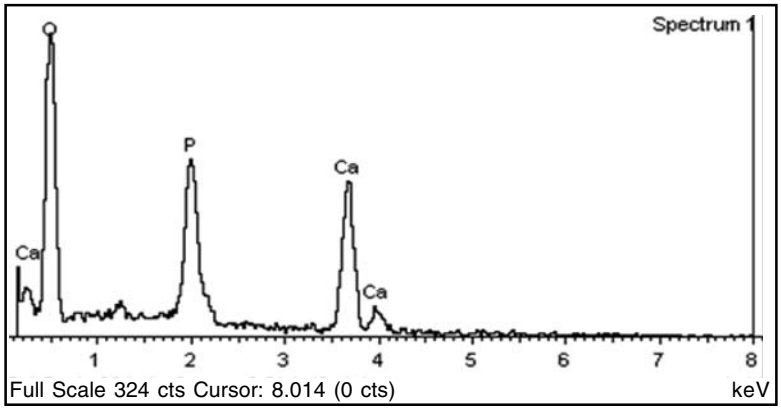


Fig. 3. EDX spectrum of coated layer

### 3.3 XRD Analysis

The XRD diffraction pattern of HA layer was shown in Fig 4. It confirms that the formation of HA with sharp diffraction at 2θ value of 25.9° and 32.62° and its corresponding planes were (002) and (211). Some amorphous peaks were also absorbed at 46.58° (222) and 49.34° (213) corresponds to the relative intensity of diffraction of standard data (JCPDS 9-0432). The major peaks and some shoulder peaks (202) plane of the layer indicate a mixture of amorphous and crystalline structure of hydroxyapatite, since the coating was carried out at low substrate temperature. Previous literature supports that better bone-tissue integration and osseointegration may be achieved due to this phase mixture in layer [28].

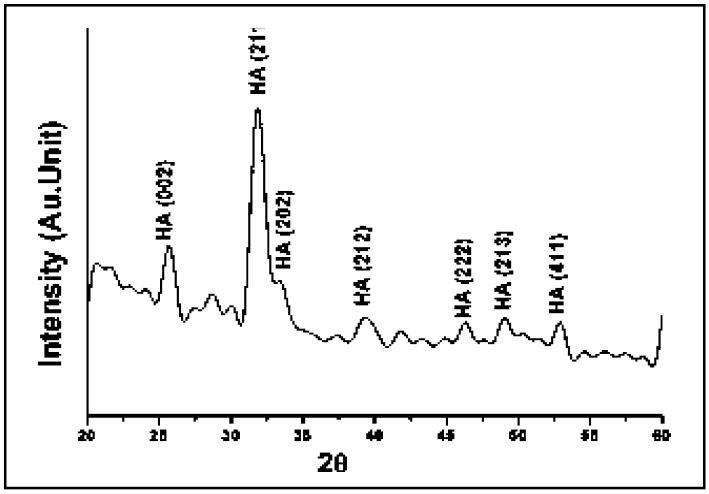


Fig. 4. XRD Pattern of HA layer

### 3.4 Adhesion Test

Mechanical properties of implants have been shown to significantly influence cell behaviors such as adhesion, growth and differentiation, and to affect the bioactivity of scaffolds used for in vivo regeneration applications of various tissues, such as cartilage, skin and peripheral nerves, also the implants requires stable adhesion strength to withstand the interlocking of bone and implants. According to ASTM F1501-94, pull-out adhesion method was used to determine the adhesion strength of the coating. The specimen which has to be tested was boned with an epoxy resin and left for 12 hours. Tensile load was applied (as shown in Figure 5) to the plane normal to the plane of coating. The adhesion strength of coating was measured till it gets peeled off from the substrate; the corresponding load determines the maximum adhesion strength of coating [29]. Figure 6 shows the adhesion strength of EB-PVD HA coating and five replicated have been carried out to avoid the random error and the average strength was found to be 14.32 ±2 MPa. Most of the samples appear to be peeled off in the glue region itself and some got struck with polyamide substrate (cohesive failure) (Figure 6), which may be due to the agglomeration of particles and leads to long penetration of glue inside the coating [30].

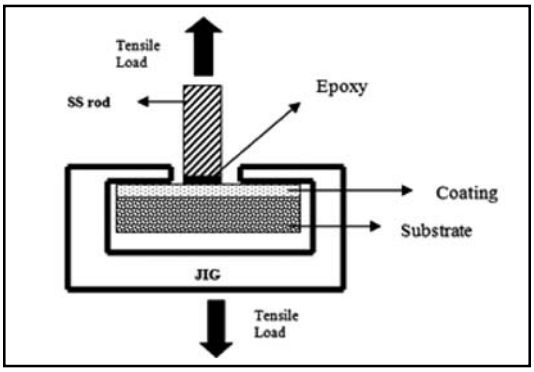


Fig. 5. Schematic of Tensile Adhesion test

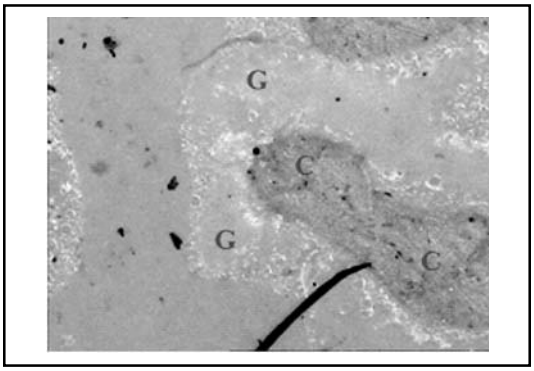


Fig. 6. Microstructure of Fracture Region

### 3.5 *in vitro* Cell Response

The *in vitro* analysis of HA coated substrates was carried out by measuring the viable cells seeded on the substrates after particular time interval 1st, 7th and 15th day. Figure 7 shows that the cells can grow more effectively on the coated. From the 1st day observation the cell growth on coated substrate was slow due to low cell-substrate interaction. The amount of cells on the coated substrate was increased

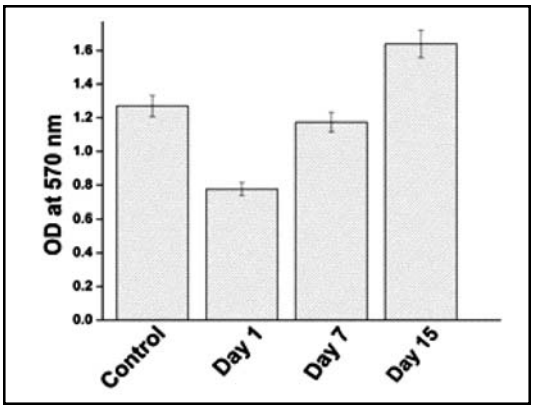


Fig. 7. MTT assay of live cells

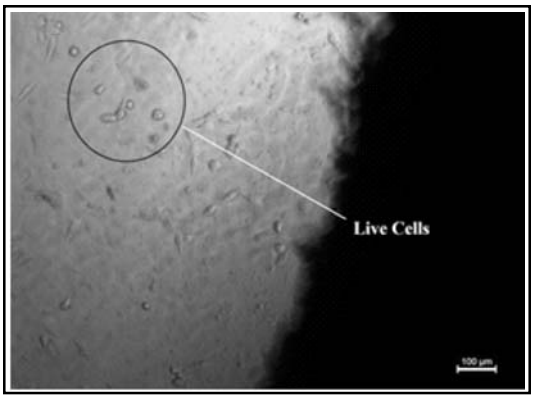


Fig. 8. Morphology of Live cells

significantly for longer incubation time. This increment cell viability was due to release of $Ca^{2+}$ ions, surface roughness and crystallinity of HA layer [31, 32] and also it was believed that the cell-substrate interaction was directly related to that of organic and inorganic interface. Microscopic image of cells seeded on coated sample was shown in the Fig. 8 it was notified that the viable cells were spindle in shape which is the basic shape of this kind of bone cells.

## CONCLUSION

In this work, synthesised HA powder was coated over the laser sintered polyamide 12 substrates using EB-PVD. The surface morphology, elemental composition, crystallinity and Adhesion strength were studied. Osteosarcoma MG-63 cells were seeded on the HA coated substrate to evaluate the cell viability. The results show that a dense layer without any cracks or pores of HA was deposited along with some agglomerated particles. There is no significant difference in physical properties of PA12 substrate after coating. This particle will increase surface roughness of the layer and promote osseointegration. Also, the Ca/P ratio of the layer was 1.68 which was close to that of standard Hydroxyapatite. Due to low deposition temperature, the coating consists of mixture of amorphous and crystalline phases. The HA layer shows very good adhesion strength towards the surface due to the obtained mirostructure. *in vitro* results reveal that the layer has better cell viability. It concluded that EB-PVD is a promising technique to deposit hydroxyapatite powder over polyamide substrate. It is more effective in improving the cell viability and has implication for enhancing the quality of biomaterials including dental and other bone related implants.

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