Effect of Particle Size on Adhesion Strength of Bovine Hydroxyapatite Layer on Ti-12Cr Coated by using Electrophoretic Deposition (EPD) Method

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Abstract. Hydroxyapatite (HA) extracted from bovine bones (called as natural HA) was used to coat a relatively new developed titanium alloy, Ti-12Cr, by using the electrophoretic deposition (EPD) method. This is to improve biocompatibility and bioactivity properties of the material to achieve optimal osseointegration in orthopaedic implant applications. There are three particle sizes of the natural HA used in this study (25 µm, 63 µm, and 125 µm) which aims to determine their effect on morphology, structure, and the strength of the resulting coating adhesion. The coating process was carried out at a voltage of 5 Volt for 5 minutes. The resulting layer morphologies and surface coverage were observed using an optical microscope. The increase in sample mass was measured using digital scales to determine the amount of the particles deposition. The coating thickness was measured using coating thickness gauges, and adhesion strength the coating layer was measured by using the cross-cut tape test method. The results of this study indicate that the HA particle size influences significantly on the quality of the coating produced after the EPD process. It is found that the coated Ti-12Cr with a small particle size has better surface properties as compared to the coarse one. Therefore, small size natural HA particles seem more suitable for implant applications.

1. Introduction
The use of hydroxyapatite (HA) coating on the surface of metals especially titanium alloys has been attracted some researchers in order to improve the biocompatibility and bioactivity properties of the material for orthopedic implants [1]–[3]. This is due to the inert nature of titanium that is unable to produce adequate osseointegration during implantation, and it is not optimal corrosion resistance in
human body environment [4], [5]. One of favor method for coating titanium is Electrophoretic Deposition (EPD) due to its simplicity, low cost and able to coat any shape. In the coating process using the EPD method, many factors will affect the quality of the coating layer. These factors include the value of the voltage used, the duration of coating, the concentration of the suspension, the pH of the suspension, the temperature of the suspension, the crystallinity of HA, the presence of the dispersant, and the particle size of the HA used [6]–[8]. The HA particle size used is believed to affect the quality of the resulting coating because it is related to the ability of deposition and the level of adhesion between the particles and the metal substrate. Furthermore, HA particle size will also influence the immune response generated by the body after implantation [9]. So, it is necessary to investigate the effect of particle sizes on the surface properties of coating layer. There are three sizes of HA particles used in this study which aims to see their effect on the morphology, structure and the adhesion strength of the coating layer.

HA particles used in this study extracted from bovine bones. The use of bovine bone HA aims to find an alternative material that is cheaper and easier to obtain compared to the commercial one that is relatively more expensive and it is difficult to have in developing countries. The important things such kind natural HA has biocompatibility and bioactivity levels that are not inferior to the commercial one [10]–[12]. The use of the bovine bone HA is also expected to provide a solution in the utilization of waste bones at the slaughtering livestock.

EPD is a technique that utilizes an electrophoretic mechanism to move charged particles suspended in a solution under the influence of an electric field to be deposited on the substrate to form a thin or thick layer. This method has potential to be used in both small and large scale industries [13]. EPD has two anodes that is anode and cathode to connect electric current. Cathode acts as working electrode where the sample is located to connect electric current. The charged HA particles are scattered in the ethanol liquid and move towards the working electrode due to electrophoresis, then deposits into sample surface due to coagulation of the HA particles [13]. A new type of titanium alloy that is claimed as low cost titanium alloy, Ti-12Cr, is used as the sample. This alloy has been relatively new developed for spinal implants due to its ability for changing modulus of elasticity.

2. Materials and methods

2.1. Sample Preparation
The sample used was a plate-shaped Ti12Cr titanium alloy with a size of 10 mm x 10 mm. The sample was then grinded slightly with sandpapers to remove any dirties that might have stuck to it during the storage period. After that, the sample is solution treated to remove residual stresses and restore the β phase of the material. After solution treatment, the sample is grinded again to remove the crusts formed during heat treatment by using #100, #600, #1000, and #1500 emery paper, until the surface of the sample is smooth and shiny.

2.2. Preparation of HA suspension
The HA powders used for coating material is derived from previously prepared bovine bones. There are three group of particle sizes of HA used in this study, that is 25 µm, 63 µm, and 125 µm which aims to determine their effect on morphology, structure, and the strength of the resulting coating adhesion. HA suspension was then prepared by mixing 4 g of HA powders into 100 mL 96% ethanol which is then stirred using a magnetic stirrer for 60 minutes for homogenization. The acidity level of the suspension was kept at pH 4 by adding 25% HNO3.

2.3. HA coating using EPD
The coating process was preceded by pre-treatment of the Ti12Cr sample. Pre-treatment is done by immersing the sample using 96% ethanol and ethanol in the ultrasonic cleaner sequentially for 15 minutes. After that, it was continued by immersing the sample in 25% HNO3 for 30 minutes followed
by soaking in NaOH for 60 minutes. The sample was then dried on a hot plate magnetic stirrer at 50°C for 5 minutes. The weight of sample was conducted before coating process.

The coating process is carried out using the EPD apparatus by attaching the sample to the cathode, in this apparatus the anode used is a carbon rod. The cathode and anode are connected to the power supply which is then set at a voltage of 5 volts, the sample and carbon rod are then immersed in HA suspension and spaced about 10 mm. The coating time used was 5 minutes. The sample was then sintered with a temperature of 800°C and a holding time of 10 minutes in a vacuum furnace. The mass of the sample was then weighed after sintering to determine the difference between mass before and after coating process. The thickness of the HA layer was measured by using the coating thickness gauges. The surface coverage was calculated after taking photos using an optical microscope. The micrograph was then processed using ImageJ software. The adhesion strength of the HA layer was measured using the cross-cut tape test method.

2.4. Data Analysis
The qualitative data were determined from the images of the HA coating layer on Ti12Cr surface. The obtained data were the increase in sample mass, HA layer thickness, HA layer surface coverage, and the percentage of damage to the HA layer after the cross-cut tape test. ANOVA test was conducted to determine the difference in the average value of each quantitative data in each HA particle size group. Statistical tests were performed using R statistic software.

3. Results and discussion
3.1. Morphology of the HA layer on the Ti12Cr surface
Figure 1 shows the morphology of the HA layer on the Ti12Cr surface after the coating process. These images show that the difference in the size of the particles used to coat Ti12Cr resulted in different layer morphologies. HA with an average particle size of 25 µm showed a much better coating ability as compared to HA powder with larger particle size. HA powder with a particle size of 63 µm looks like quite difficult to coat on the Ti12Cr surface because there were still many empty gaps that had not been filled. Meanwhile, the powder with a particle size of 125 µm seems unable to coat properly on the surface of the material. There were only a few particles attached.

Coating using the EPD method utilizes electric current as energy to deposit coating particles on the surface of the substrate to be coated. The most influential factors in this process are the voltage and coating time [14]. Meanwhile, in this study, the voltage and time used were constant at 5 volts and 5 minutes and the coating powder used had different particle sizes. The results of this study indicate that the smaller particles look easier to coat the surface of the Ti12Cr substrate by utilizing the available energy. The results of this study also confirmed that the trend for the coating process that continues to use coating particles that are smaller and even up to the nanoscale is because it will produce a much better layer morphology [15].

![Image of HA layer morphologies](image-url)  
**Figure 1.** Morphology of the HA layer on the Ti12Cr surface, (a) 25 µm particles, (b) 63 µm particles, and (c) 125 µm particles.
From figure 1, it can be seen that HA powder with fine particle size produces an even layer morphology and there are no visible gaps or particle buildups. Meanwhile, the powder with a particle size of 63 µm was not able to coat evenly because there were many empty gaps and there was a lot of uneven particle buildup on the surface of the Ti12Cr substrate. Furthermore, there is another assumption which states that it is difficult for large particles to coat the surface of the substrate due to the small tangential contact that occurs between each HA particle which results in weak bonds formed so that the HA particles are easy to release and do not completely coat the substrate surface [16].

3.2. Mass Growth of Samples

Figure 2 shows the effect of particle sizes on the mass growth of the sample (or additional mass) after coating with the natural HA powders. It can be seen that the highest layer mass is found in samples with HA particles measuring 63 µm, while samples with HA particles measuring 25 µm and 125 µm did not appear to have significant layer mass and tends to be very low. This difference is probably due to differences in the ability of energy from electric currents to deposit HA particles. Logically, particles with smaller sizes will be easier to be positioned on the surface of the substrate compared to larger particle sizes because the energy required is also smaller. However, in this study, there was an oddity because samples coated with HA with a particle size of 63 µm had a much higher mass as compared to samples coated with HA with a particle size of 25 µm. While samples coated with HA with a particle size of 125 µm tended to have mass relatively low. This is probably because HA particles with the size 25 µm do have a much lower mass than particles with larger sizes so that when measured using a balance, the resulting mass is much lower even though the HA particles have covered the entire surface of Ti12Cr (figure 1 (a)). Meanwhile, the HA particles measuring 63 µm had a higher mass when measured even though the entire surface of the Ti12Cr sample was not completely coated (figure 1 (b)). Based on these results, it can be ascertained that the increase in sample mass cannot be used as a single reference to assess whether the coating process has given the desired results because the high mass gain does not necessarily indicate good or perfect coverage even though the mass increase can be used as a reference for the occurrence of HA particle deposition processes on the substrate to be coated.

![Figure 2](image)

**Figure 2.** Relationship between HA particle size and the increase in sample mass.

The statistical tests performed using the ANOVA test, shows that each sample group with different HA particle sizes do not have a significant difference in mass gain (F-value = 2.54; p-value = 0.15). Thus, it could be concluded that HA particle size do not give a significant effect on the mass increase of the sample. A study shows that voltage greatly affects the deposition of HA particles onto the surface of titanium, coating carried out at constant voltage will deposit large particles and the formed layer will be porous, while dynamic voltage will produce a layer that has a gradient from large particles to large particles. small size [6], [7]. Other studies have also stated that the particle size
greatly affects the quality of the HA layer produced by the EPD method because it greatly affects the mobility of charged particles in the coating process [2].

3.3. HA coating thickness

Figure 3 shows the relationship between particle size and the HA coating thickness for the indicated particle size. It can be seen that there is no significant difference in layer thickness between each HA particle size group. The HA particle size used as a coating does not have a significant effect on the coating thickness. In this study, the voltage and coating time given are constant at 5 volts and 5 minutes so that the amount of energy and time given to each sample is the same as the expectation that the particle size used will make a difference, but the particle size of the coating layer does not give a significant effect on the thickness of the layer.

However, other assumptions can be used to explain this phenomenon. In the coating process using the EPD method, the layer formed consists of small particles that are arranged homogeneously and evenly layer by layer. The thickness of the HA layer formed will certainly be the same as the diameter of the coating particles or their accumulation if the particles accumulate to form layers. Thus, the layer formed by particles with a size of 25 µm requires more than four layers to reach a thickness of more than 100 µm while HA particles with a size of 125 µm only need one layer to achieve the same thickness. Thus, although the deposition of HA particles with a size of 125 µm tends to be lower, they can have the same thickness due to the particle size itself.

![Figure 3. Relationship between HA particle size and the coating thickness.](image)

The results of statistical analysis using the ANOVA test also shows the same conclusion (F-value = 0.56, p-value = 0.59), namely that there is no significant difference in layer thickness between the groups of HA particle size used as a coating material. The layer thickness is very much influenced by the dynamics of the applied voltage [8]. The higher the applied voltage, the thicker the resulting layer will be, and vice versa, the lower voltage will produce a thin layer [17]–[19]. The thickness of the resulting coating is not much difference between each treatment group since the voltage is constant at 5V.

3.4. Surface Coverage of HA coating

Figure 4 shows a graph of the relationship between the HA particle size used in the coating process and the surface coverage that produced. It can be seen that there is a significant difference between the 25 µm particle size group and the other groups. The results of statistical tests using the ANOVA test also shows that there is a significant difference in the mean surface coverage values generated between the particle size groups (F-value = 4.34; p-value = 0.06). These results indicate that the smaller particle size is easier to form severe layer than the larger particle size.
Figure 4. Relationship between HA particle size and surface coverage.

The availability of energy produced by a voltage of 5 volt appears to be able for distributing HA particles with a size of 25 µm across the surface of the Ti12Cr substrate as evidenced by the surface coverage value that can exceed 80%. However, for HA particles with a larger size, it looks very difficult to be positioned using the available energy. It is necessary to know proportional particle size so that the resulting layer is better. In connection with the results of this study, it can be assumed that 25 µm is a proportional particle size for use. So far it has been known that the voltage and coating time are important factors affecting the surface coverage ability of HA particles on the surface of titanium implants [14], [15], [17]–[20]. Besides, the HA particle size seem also giving an equally important effect.

3.5. Damage Percentage of HA layer after Cross-Cut Tape Test

Figure 5 is a graph the relationship between the HA particle size used as a coating material to the percentage of damage produced after the Cross-Cut Tape Test. It can be seen that there is a significant difference in the percentage of damage between each group of particle size. The highest damage was found in the group of particles with a size of 125 µm and the lowest one is in the group of particle sizes of 25 µm. The results of statistical tests using the ANOVA test also shows the same conclusion, there is a significant difference in the percentage of damage between each treatment group HA particle size (F-value = 354.44; p-value = 5.91 x 10^-7). This result indicates that the smaller the particle size will increase the adhesion strength of the HA particles to the surface of the Ti12Cr substrate. One of the assumptions that can be used to explain this result is that the smaller particles will have a greater tangential contact between the particles so that the bonds formed are also stronger when compared to larger particles. Logically, HA particles with a large size will have a bond with the surrounding particles but also have gaps that are large enough on the non-bonded side, whereas in smaller particles the gap will also get smaller so that the contact that occurs is much more area and the bonds formed are getting stronger. One study stated that the adhesion strength of HA particles on the surface of titanium is influenced by the uniform distribution of porosity [21]. Samples coated with HA particles of 125 µm and 63 µm have a low surface coverage value that means they also have a low porosity distribution, thereby reduces adhesion strength. Residual stress also has a major effect on the weakening of the adhesion of HA particles on the titanium surface. The easy release layer shows that the residual stress on the surface of the material is also quite high [22], [23].
4. Conclusions

In order to improve surface properties of new-developed titanium alloys, Ti-12Cr, a bovine bone HA has been coated to the metal surface by using EPD method. There are three group of particle sizes this kind natural HA used in this study, that is 25 µm, 63 µm, and 125 µm. The coating process was carried out at a voltage of 5 Volt for 5 minutes. The physical and mechanical properties of the coating layer were then examined and tested, respectively, using adequate equipment. The following conclusions are obtained:

1. Particle size has a significant effect on the surface performances including mass growth and surface coverage, but almost no effect on the thickness of coating layer.
2. Adhesion bonding of the coating layer resulting from a small particle size is much better than that of the coarse one.
3. Natural HA coating layer with the size of 25 µm or less is recommended to use in order to improve significantly biocompatibility and bioactivity of Ti-12Cr.

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