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Electrophoretic deposition of HA/MWNTs composite coating for biomaterial applications

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Abstract A composite coating of hydroxyapatite (HA)/multi-walled carbon nanotubes (MWNTs) has been fabricated by electrophoretic deposition (EPD). The nano powders of HA and MWNTs were dispersed in ethanol with total concentration of 0.005 g/mL and MWNTs 20% and 30% contents (wt). And the pH value of suspension was adjusted in a range from 4 to 5. After stabilization the mixture was ultrasonically treated for 3 h to form a stable suspension. Prior to the electrophoretic deposition, the titanium substrate was hydrothermally treated at 140 in NaOH (10 mol/L) solution for 6 h. A titanium sheet and circinal net of stainless steel were used as a cathode and an anode respectively, and a constant deposition voltage of 30 V was applied for 50–60 s in the EPD process. The thickness of the coatings was controlled from 10 μm to 20 μm. The samples of composite coating were then sintered in a resistance tube furnace in flowing argon at 700 for 2 h. The structure of the as prepared coating was characterized by SEM and XRD, and the bonding force of the coating/substrate was measured by an interfacial shear strength test. It is shown that the bonding strengths between the coating and the titanium substrate is as high as 35 MPa. The cell culture experiments indicate that the prepared composite coating of HA/MWNTs possesses good biocompatibility.

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Introduction

Hydroxyapatite (HA) formulated as $Ca_{10}(PO_4)6(OH)_2$ has been extensively studied because it is a most important mineral component in natural bone and teeth, and its synthetic form is capable of forming a direct chemical bond with bone. HA has osteoconductive and bioactive properties that is very essential for rapidly promoting bone formation and strong biological fixation to bony tissues [1–3]. Unfortunately, the poor mechanical properties of HA devices severely inhibit its clinical applications for high load-bearing conditions. As a result, the significant research activity has been associated with the development of HA coatings on metals or HA composites, including the HA fibers and whiskers [4–6]. Titanium is most popular to be used as a metal substrate for HA coating, because of its similar mechanical properties to bone, and also high corrosion resistance. Many processing methods have been developed to coat HA on metal substrate, to enable the HA/metal composite to have a suitable mechanical properties and good biocompatibility. They include plasma spraying [7], electrochemical deposition [8], sol–gel coating [9, 10], and biomimetic coating [11] etc, but each of them has shortcomings such as the decomposition of HA result from extremely high processing temperature, or weak adhesive to substrate, or crack of the coating. Electrophoretic deposition (EPD), as a coating technique, has been widely applied in the labs and fields due to its efficiency, flexibility, economy and easy control of thickness (from 1 μ to 500 μ) [12, 13]. Because the thermal expansion coefficient is not well mismatched between the titanium substrate and the HA coatings, a residual stress generally remains in the coating resulted from the sintering process, and it may reduce the adhesion between the coated HA layer and substrate [14]. Many studies of improvement of mechanical

property for biomaterials by developing composite or grads coatings have been carried out [15, 16]. In this work we intend to solve the problem of the mismatch of thermal expansion coefficient between titanium and HA coating by EPD composite coating on titanium.

Since the discovery by Ijima in 1991 [17], carbon nanotubes (CNTs) have attracted tremendous attentions. It is indicated that CNTs possess excellent mechanical properties, electronic properties and chemical stability, due to their cylindrical graphitic structure [18]. It is well known that carbon is one of known fundamental elements in the development of life on the planet earth [19]. There are two kinds of nanotubes, single-walled nanotube (SWNTs) and multi-walled nanotube (MWNTs). Based on the Hall-Petch principle, the smaller the particles reinforced in the coating, and more strengthened the coating will be [20], and the addition of MWNTs is able to transfer and eliminate residual stress in the coating, in present work, we aimed to make HA/MWNTs composite coating using EPD followed by sintering in high purity argon gas. Though the similar composite coatings on titanium have been documented, the rigor experiment condition prevents it from widespread use, and also the decomposition of HA due to extremely high temperature may affect the biocompatibility [21]. And the composite block devices of HA/MWNTs have also been reported in the literatures [22–24], however it is far from perfect to put into real application. In this wok we try to develop a simple method for fabricating HA/MWNTs composite coating on titanium with both a better mechanical properties and excellent biocompatibility.

Experiment

The HA powder with an average particle size ranging from 30 nm to 50 nm was used in this work. Nano sized powder of HA was hydrothermally synthesized in our lab based on the following reaction [4]:

$$10Ca(NO_3)_2 + 6(NH_4)2HPO_4 + 8NH_4OH = Ca_{10}(PO_4)_6(OH)_2 + 20NH_4NO_3 + 6H_2O$$

MWNTs was provided by College of Chemistry & Chemical Engineering at Xiamen University. It was firstly brought to a reflux in a concentrated nitrate acid for 4.5 h and then filtered over a 1 μm PTFE membrane filter with copious amounts of water and ethanol. Ethanol was used as medium for EPD. Commercially pure titanium sheet with size of 10 mm × 20 mm × 2 mm was used as substrate. Prior to the deposition, titanium substrates were firstly polished by sand paper to remove the surface oxide film and then were ultrasonically rinsed in ethanol and acetone and washed by DI water. After that the titanium sheet was hydrothermal treated at 140 °C in NaOH (10 mol/L) for 6 h, and then immersed into 5% $NaHCO_3$ for more than 10 h to moderate the surface of substrate. A circinal net of stainless steel (SS) with diameter of 50 mm was used as an anode. The titanium substrate was put into the inside of the circinal stainless steel as cathode where deposition occurred. Figure 1 is an experimental set-up for the EDP process. As we know, electrophoresis essentially involves the migration of charged particles towards the corresponding charged electrode, from which the deposition occurs. MWNTs can be positively or negatively charged with the action of cationic or anionic surfactants [25], so the process of EPD for CNTs can occur [26–29]. The measurement of Zeta potential showed that the particles in this suspension acquired a positive charge and so their deposition occurred at the cathode of Ti substrate. This experiment arrangement has advantages to efficiently get very smooth coating without crack in this deposition.

The HA and MWNTs powers were mixed with MWNTs 20% or 30% content (wt). Then the mixture was dispersed in ethanol for the total concentration 0.005 g/mL, and nitrate acid was used for adjusting the pH value of the suspension ranging from 4 to 5. It was aged for 1 day to stabilize the suspension solution. After that the mixture was ultrasonically treated for 3 h to obtain a dispersed suspension. After ultrasonic treatment, the medium showed a great impact on the dispersion of MWNTs. Then the suspension was centrifuged at 2,000 rpm for 2 min to remove any large particles. The supernatant was decanted and prepared for further use. Before deposition the suspension was ultrasonically treated again for another 0.5 h. Then the alkali treated titanium sheet was immersed in the electrophoretic tank with the former prepared suspension. The EDP process was carried out at room temperature. The thickness of growing layer of composite coating of HA/MWNTs on titanium was controlled by the current parameters and time of deposition. A deposition voltage of 30 V and a deposition time of 50 s were selected to achieve

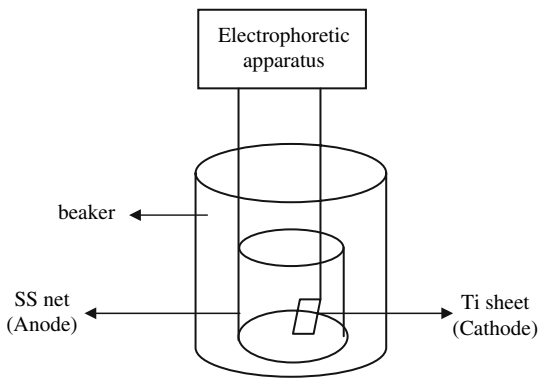

Fig. 1 Illustration of set-up for electrophoretic deposition

the required thickness of the coating (nearly 10 μm in this work). After finished the deposition, the sample was removed carefully from the EDP cell and dried in the air, and then sintered at 700 °C for 2 h in a flowing high purity argon with the rate of 5,100 mL/h.

The surface morphology of the composite coatings after sintering was observed by a scanning electron microscope (FEI XL30 ESEM-TMP and FESEMLEO 1530). X-ray Diffraction (XRD) analysis was used for phase analysis of the composite layers (RIGAKUD/MAX-RC). An epoxy resin was glued on the full top of coating and cured at 165 ± 1 °C for 1.5 h in an oven. The adhesive strength (AS) between coating and substrate was measured with a universal testing machine (DWD-100) using a 10 kN load cell and speed of 1.0 mm/min. The end of one sample was fixed, while the other end of it was given downward pull (as shown in Fig. 2). Further the data analysis of this test method was easily calculated from the applied drawing force over the fractured area (1 cm^2 approximately) [30–32]. Each data of the adhesive strength was an average value from the three samples prepared and measured in the same conditions.

Another type pure titanium sheet with size of 10 mm × 10 mm × 2 mm was also used as substrate for composite coatings. The specimens made as described above were then used for cell culture in vivo to testify its biocompatibility. Osteoblast-like MG 63 cells were used for this research to imitate the behavior of osteoblasts because this cell line possesses the osteoblast phenotype. Culture medium was RPMI1640 (containing 15% fetus cow serums, the penicillin 100 Us/mL, streptomycin 100 μg/mL) and MG63 cells were cultured on the specimens (5 × 104 cells/cm^2 in density) after 3 day of incubation in carbon dioxide culture box (5% CO_2, 37 °C, saturated degree of humidity) [14]. Fixed with 2.5% fixing solution 2 h at 4 °C, the specimens was then cleaned with 0.1 M phosphate buffer solution (4 °C) three times for 15 min every time. After dehydration the specimens were dipped in the isoamyl acetate and dried at a critical temperature. Then it was gilded and observed by SEM (XL30E).

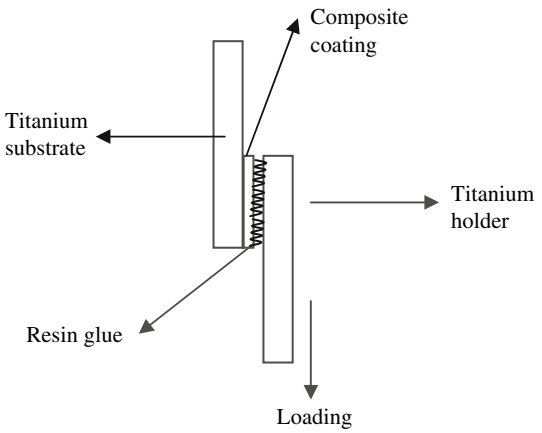

Fig. 2 Schematical arrangement of mechanical test for the adhesive strength between the coating and substrate

Results and discussion

In order to get a stable suspension, many kinds of medium have been developed. Ethanol was chosen as the dispersant in this work because of its suitability for HA EPD and innocuity to environment. It was noted that the EPD deposition of composite coating should be at cathode, otherwise the high voltage during the process of EPD may result in a severe oxidation of titanium. So the particles of HA and CNTs in the suspension should be positively charged to enable their migration towards the cathode under the applied field. Accordingly, we adjusted the pH value for the suspension to have the particles in positive charge by adsorbing H^+ and other positive charges. It was found that the EPD behavior of the HA particle was greatly influenced by the pH value of the suspension. The HA particles were stable in an alkaline environments, but partially or totally dissolved in acidic electrolytes depending on the degree of acidity. It was indicated that the HA particles might start to dissolve when pH value was under 4. So the pH value of suspension from 4 to 5 was made as our best choice in the experiment. While H^+ also benefited to assist MWNTs in dispersion. We finally got a stable and well dispersed suspension after ultrasonication, centrifugalization and another ultrasonication. The zeta potential was measured and it showed that both HA and MWNTs particles had average size of 200–300 nm and they all in positively charge. The sintering process was controlled with 4 °C/min in ascending of the temperature, keeping 2 h at 700 °C, and then 1.5 °C/min in descending of the temperature. It is noted that the nano sized HA and MWNTs are enabled the composite coating to achieve a high density at a sintering temperature as low as 700 °C [33]. After sintering the coatings on pure titanium appeared smooth and crack-free.

The SEM observations were conducted to confirm the existence of MWNTs in the composite coating after sintering at high temperature. Figure 3a and b shows the morphologies comparisons for the composite coatings after and before sintering at 700 °C for 2 h with MWNTs 20%(wt) contents. Figure 3c and d are SEM image comparison of the coatings after and before sintering in at 700 °C for 2 h with MWNTs 30%(wt). It is shown that the MWNTs retain after sintering and distribute uniformly in the composite coating. The MWNTs in the coating play a crucial role in reinforcing phase and enhancing the mechanical properties by interleaving conjunction with

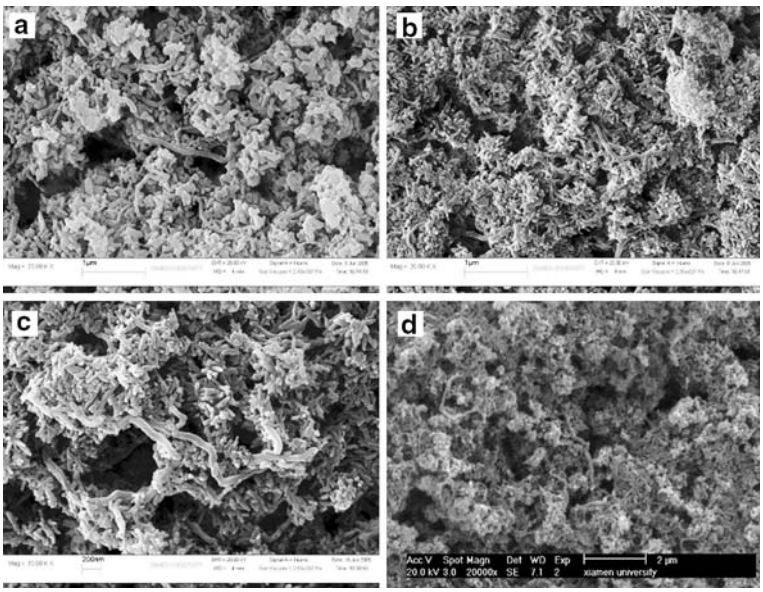

Fig. 3 Micrograph comparison of coatings before and after sintered with different MWNTs contents. (**a**) Coatings after sintering at 700 °C with MWCNTs 20%; (**b**) coatings before sintering at 700 °C with MWCNTs 20%; (**c**) coatings after sintering at 700 °C with MWCNTs 30%; (**d**) coatings before sintering at 700 °C with MWCNTs 30%

HA. It is generally recognized that the smaller the particles reinforced in the coating, and more strengthened the coating will be. Additionally, the addition of MWNTs is able to transfer and eliminate residual stress in the coating through its microstructure improvements. And it is obvious that the MWNTs with their original tubular structure can be observed after sintering and the claviform HA crystalloid also can be seen before and after sintering. It is indicated that low sintering temperature is very desirable to prevent from decomposition of nano HA, and sintering in an argon ambience is also very necessary to remain MWNTs structure in the composite coating.

Figure 4a and b shows XRD spectra for the composite coating with 20% MWNTs content before and after sintering respectively. No other crystalline peak is observed except the diffraction peaks attributed to the MWNTs and HA. Figure 4b has even sharper peaks indicating that sintering is possible to make HA crystal more perfect. The MWNTs have the same XRD peaks as graphite. In Fig. 4a the peak at $2\theta = 26°$ is the graphite (002), which also appears in Fig. 3b in the same intensity indicating that the MWNTs almost keep the same content in the composite coating during sintering process. Figure 4c and d shows XRD results for the composite coating with 30% MWNTs content before and after sintering treatment. They have a similar crystal structure comparing to the coating with 20% MWNTs. The X-ray diffraction patterns of the composite coating before and after sintering indicate that the sintering at 700 °C in argon atmosphere does not change the composition of the coating.

The bonding strength between the coating and the titanium substrate was measured by a shear strength test. The typical result is listed in Table 1. It is found that the adhesive strength of the composite coating to titanium

Table 1 Adhesive strength between the composite coatings and titanium

Samples	Composite coating with 20% MWCNTs	Composite coating with 30% MWCNTs	Pure HA
Bonding strength	34.94 MPa	35.44 MPa	20.62 MPa

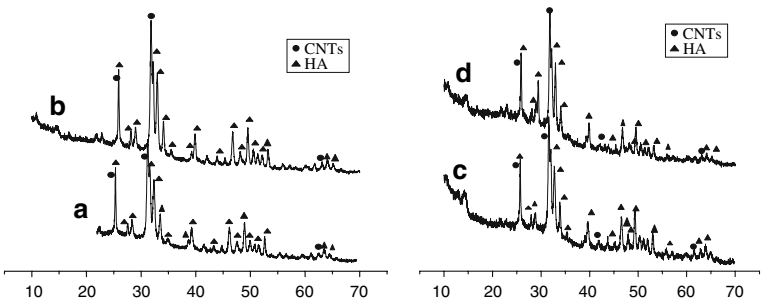

Fig. 4 X-ray diffraction of coatings before and after sintered with different MWCNTs contents. (**a**) Composite coating before sintering with MWCNTs 20%; (**b**) composite coating after sintering at 700 °C with MWCNTs 20%; (**c**) composite coating before sintering with MWCNTs 30%; (**d**) composite coating after sintering at 700 °C with MWCNTs 30%

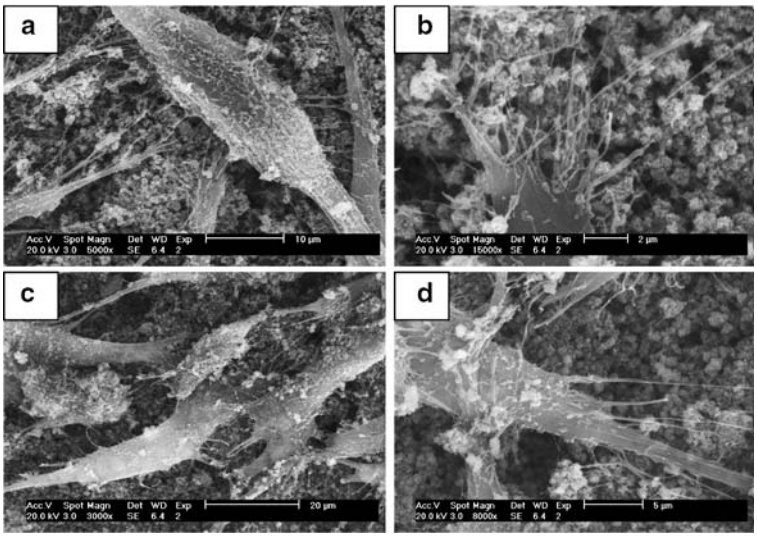

Fig. 5 SEM images of MG63 cells cultured on the composite coating with 20% MWCNTs contents. The composite coating before sintering (**a**, **b**), and the composite coating after sintering (**c**, **d**)

substrate is much stronger than that of the pure HA coating. And with increasing of MWNTs from 20% to 30% in the coating the adhesive strength becomes higher, but this increase of bonding strength is not very much. It is also believed that the bonding strength between MWNTs and HA is strong enough in the composite coating because a chemical bonding of HA on MWNTs surface may form in the sintering condition. It has been reported that the measured shear strength of natural cortical bone is 35 MPa [14]. The bonding strength of the prepared composite coating to substrate approaches to this value, so the composite coating prepared in this work is of acceptable mechanical properties for using in high load-bearing conditions as an implant biomaterial.

The biocompatibility of the prepared composite coating was evaluated by in vitro cell culture. Figure 5 shows the typical SEM micrographs of the attached MG63 cells on the surface of the composite coating of MWNTs/HA after 3 days of cell incubation. It is indicated that the inoculated MG63 cells attach and grow well on the surface of MWNTs/HA composite coating. A closed interaction of cell and the MWNTs/HA composite coating is discerned, meaning that the prepared composite coating presents a high affinity to the cells incubated on it. All the experiments of cell culture has demonstrated that the composite coatings are not only non-toxic effect to the MG63 cells, but also exhibit a good biocompatibility, which is probably attributed to the extreme high surface area and special porous structure for the MWNTs/HA composite coating.

Conclusion

An EPD has been developed to prepare the nano composite coating of HA/multi-walled carbon nanotubes (MWNTs). It is found that the nano composite coating fabricated by EPD and sintered in a resistance tube furnace in flowing argon at 700 °C for 2 h is of a compact surface and crack free, and the MWNTs are well dispersed in the composite coating. The measurement of adhesive strength between coating and substrate exhibits a great mechanical property up to 35.4 MPa, approaching to that of the natural bone. The in vitro cell culture has demonstrated that the composite coatings are not only non-toxic effect to the MG63 cells, but also exhibit a good biocompatibility. It is reasonable to believe that the nano composite coating of HA/MWNTs is promising to be applied for high load-bearing conditions in human body in the future.

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