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Modifying of the Biocompatible HA/Mwcnts/BSA Composites with TiO_2 for Using as a Bone Replacement Materials

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Bone is a natural nano-structured composite composed of organic compounds, which is mainly collagen, reinforced with inorganic compounds like hydroxyapatite (HA) [1]. HA is very similar in composition in the mineral phase of bone; it has good biocompatibility *in vitro* and *in vivo* and it is an excellent material for use in bone replacement purpose [2]. However, due to its low tensile strength and brittleness, HA alone could not make up as great bone replacement material due to its poor mechanical properties. In order to increase the mechanical strength of HA, variety of reinforcing elements ranging from particular bioceramic inclusions to polymer fibers and carbon nanotubes (CNTs) have been considered [3]. The reinforcement could significantly affect the mechanical strength and toughness of the bone replacement material [4,5].

Since CNTs entered the world materials stage, their mechanical properties have been praised as some of the best present. Their strength and stiffness, combined with their small size and large interfacial area, suggest they may have great potential as reinforcing agent for HA. CNTs are a promising new material owing to its unique internal structure, low mass density, remarkable chemical stability and electronic conductivity [4,5]. The addition of metal nanoparticles to organic materials is known to increase the surface hydrophobicity and to reduce adherence to biomolecules. By using titanium dioxide (TiO_2) as an additional component in the composite, it is expected to improve mechanical strength and antimicrobial properties of the composites.

Coating of stronger material on the CNTs has been introduced to enhance the mechanical strength of CNTs. Basically, CNTs coated with metal oxides are expected to exhibit different or better physical properties than those of neat nanotubes. Metal is chosen to be coated with CNTs due to its superior mechanical properties, which allow for load-bearing situations [6]. CNTs and TiO_2 composite materials are attractive materials for researchers in relation to the treatment of contaminated water and air by heterogeneous photo-catalysis, hydrogen evolution, CO_2 photo-reduction, and dye sensitized solar cells. There are many methods could be employed to coat the TiO_2 such as plasma spraying, electrophoretic deposition (EPD) and sol-gel. Plasma spraying technique is widely used because of its process feasibility [7]. It is ease to operation, but it state that the thick coating produced often exhibit porosity. The porosity will weaken the interfacial strength and leads to adhesion failure [7]. EPD method is a cost-effective method (low equipment costs, simplicity in setup) and able to fabricate free standing objects and coatings from particulate materials. It offers rigid control of film thickness, uniformity and deposition rate [8]. Sol-gel technique is a famous processing route in fabricating optical quality film, forming planar optical wave guides and most surface coating on glass. The inhomogeneity TiO_2 coating on the CNTs surface, the damage of the CNTs surface structure after coating and the thermal stability of the TiO_2 layer deposited on CNTs have been reported. This might be because of the deficient purification or lack of sufficient functionalization of multi-walled carbon nanotubes (MWNTs) before coating [9]. Generally, sol–gel method leads to a heterogeneous, nonuniform coating of CNTs by TiO_2, showing bare CNT surfaces and random aggregation of TiO_2 onto the CNT surface [10]. The most common precursors in TiO_2 coating is titanium tetra-isopropoxide (TTIP). It is chosen due to it can be readily dissolves in alcohol and is not overly sensitive to humidity [11]. As for the solvent, methanol had been chosen as it could condense to generate water to hydrolyze TTIP under supercritical state [12]. Basically, the formation of TiO_2 coating is by the hydrolysis-polycondensation of titanium alkoxides, which in this case is TTIP [13].

This study aims to investigate the effect of coating different types of MWCNTs (MWCNTs, Hydroxylated multi-walled carbon nanotubes (MWCNTs-OH) and Carboxylated multi-walled carbon nanotubes (MWCNTs-COOH)) on mechanical properties of the composite. Furthermore, antimicrobial properties of the composite were studied. Briefly, conventional sol-gel preparation method was performed for the coating process. Hydrolysis and condensation reactions were performed by adding in methanol and titanium (IV) isopropoxide solution. The MWCNTs were added to the solution, and then the resulting gel was filtered and dried for 3 hours at 80°C before being air-calcined in a preheated furnace (400°C, 5 hours). HA was physically mixed with BSA and TiO_2-MWCNTs. The prepared paste was packed into a cylindrical stainless steel mold (diameter=6 mm, height=12 mm) and incubated for 24 hours (37°C and 97% humidity). The disc diffusion test was carried out on nutrient agar medium by following the guidelines standardized by the National Committee for Clinical Laboratory Standards (NCCLS).

This present work demonstrated the possibility of developing high compressive strength and biocompatible composite (HA/BSA/MWCNTs) by reinforcement with coating TiO_2 on MWCNTs for use as bone replacement material. Of all the MWCNTs studied, TiO_2-MWCNTs (compare with functionalized MWCNTS) were found to be the most effective to increase the mechanical strength of the composite.

It was suggested that the defect sites on the surface of MWCNTs improved the coating of TiO_2 on MWCNTs leading to stronger composite form. TEM observations (Figure 1) demonstrated that the coating of TiO_2 on non-functionalized MWCNTs was better as compared to functionalized MWCNTs. TiO_2 layer is uniformly deposited on the pure MWCNTs. The hollow structure of the pure MWCNTs is filled and invisible after the coating. The diameter of

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the TiO_2 coated MWCNTs was estimated to be about 50-70 nm. The diameter of the pure MWCNTs is originally about 20-30 nm. Hence, a relatively 5-20 nm of TiO_2 layer was coated. Drawing on the results from the compressive strength tests, the HA/TiO_2-MWCNTs/BSA composite exhibited substantially improved compressive strength as compared to pure HA. Figure 2 shows that among the three types of MWCNTs, TiO_2 with pure MWCNTs gave the best mechanical strength. As for MWCNTs-OH and MWCNTs-COOH coated with the similar weight percentage of TiO_2, both showed quite a similar mechanical strength in this test. The mechanical strength results of TiO_2 coated functionalized MWCNTs were relatively weaker than TiO_2 coated non-functionalized MWCNTs [14].The compressive strength of the HA could not be measured because it was too weak to form the required shape for compressive test purposes. The compressive strength of HA/BSA composite is higher than pure HA, it indicates that BSA can improve the mechanical properties of the composite [9].

Basically the antimicrobial tests were done on microorganisms that can cause implant-related infection to investigate the antimicrobial activities of the samples [15]. The type of microorganism being chosen for the test was Staphylococcus aureus. It is a multipurpose and dangerous pathogen exists in human bodies [16]. This gram-positive bacterium is one of the most common microorganisms in human bodies' infection [17]. By using the published Clinical and Laboratory Standards Institute (CLSI) guidelines, we can determine the susceptibility or resistance of the microorganism to each sample tested. The antimicrobial tests showed that the samples have a little antimicrobial effect on the microorganism. All three of the samples showed similar results based on the observation made. It was suggested that the antimicrobial effect of TiO_2 was suppressed due to coating on MWCNTs, however, the thin TiO_2 biofilm managed to form a protective layer and fell under 'Resistant' category according to CLSI definition.

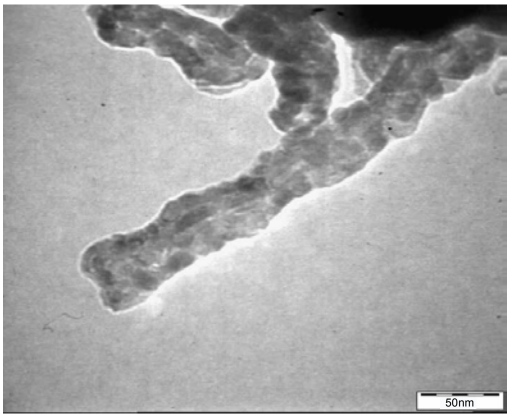

Figure 1: TEM images of 90 wt. % TiO_2 coated MWCNTs.

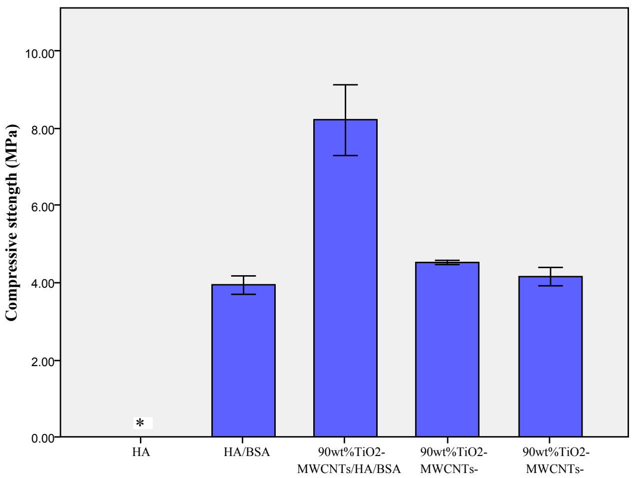

Figure 2: Compressive strength of the HA/TiO_2-MWCNT/BSA composite by using different types of MWCNTs.

Between the novel HA/TiO_2-MWCNTs/BSA composites with different types of MWCNTs (functionalized and non-functionalized MWCNTs) presented in this study, the composite which was prepared by using TiO_2-MWCNTs appeared to be most effective in term of increasing the mechanical strength of the composite. TEM observations showed that the coating of TiO_2 on MWCNTs was better as compared to functionalized MWCNTs. Diffusion disc test results showed that the antimicrobial effect of composite fell under 'Resistant' category according to CLSI definition.

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