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Apatite forming ability of Ti-HA-MWCNTs composite

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ABSTRACT

The establishment of biocomposites of titanium (Ti) with a bioactive material, like hydroxyapatite (HA), as well as multi-walled carbon nanotubes (MWCNTs), is introduced as a substitute to enhance the response in the tissue-implant interface. This research investigated the effect of MWCNTs addition on the bioactivity properties of Ti-HA composite made by mechanical alloying and powder metallurgy. MWCNTs with varying contents (5 and 10 wt.%) were prepared. After milling the powders at 200 rpm for 2 h, the composite was isostatically compacted before sintering at 1000 °C for 2 h. The bioactivity properties and its morphology were investigated using immersion test and scanning electron microscope (SEM), respectively. The results indicate that higher MWCNTs content is associated with increased apatite weight and growth. The composite containing 10 wt.% MWCNTs exhibited a weight gain of 1.72%, which was higher than that of the 5 wt.% MWCNTs composite (1.56%). A higher MWCNTs content increased apatite weight from 0.02 g (5 wt.% MWCNTs) to 0.03 g (10 wt.% MWCNTs). The composite surface with 10 wt.% MWCNTs had more pronounced apatite coverage, which was mostly made up of spherical crystals. According to this finding, the Ti-HA composite with 10 wt.% MWCNTs showed higher bioactivity, as evidenced by increased apatite formation and improved bioactivity properties.

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1. INTRODUCTION

Extensive research is underway on bio-implant materials to meet the increasing need to alleviate the damage caused by ageing, injuries, as well as diseases. Titanium (Ti) and its alloys are used as hard tissue implants to substitute and/or restore damaged or diseased tissues due to their excellent corrosion resistance, low density and high strength [1-3]. However, their inherent bio-inertness can hinder the formation of a strong bond with bone, increasing the risk of loosening or implant failure.

Consequently, patients may experience poor clinical outcomes and necessitate additional revision surgeries. The bioactivity properties have the potential to prolong the lifespan of non-bioactive implants, which is especially important for younger patients who rely on these implants for long-term functionality. The significance of bioactive implants lies in their ability to provide better support and stability to surrounding bone tissue, ultimately resulting in improved functionality and quality of life for the patient. For example, a bioactive hip implant enables patients to regain mobility and participate in their preferred activities. A common strategy for increasing the bioactivity of Ti implants is to incorporate bioactive materials such as hydroxyapatite (HA), a calcium phosphate ceramic that closely resembles the mineral constituent of bone [4]. Nevertheless, HA has some disadvantages, including fragility, limited tensile strength, and low fracture toughness [5]. To overcome these shortcomings, HA is often reinforced with ceramic materials, including multi-walled carbon nanotubes (MWCNTs) or metallic materials, like Ti to produce composites with improved properties.

Multi-walled carbon nanotubes (MWCNTs) have exceptional mechanical strength and high surface area [6]. Additionally, MWCNTs can serve as nucleation sites for apatite formation [7], encouraging the growth of apatite crystals on the composite's surface, thereby presenting a promising solution to the aforementioned challenge. This is supported by a study performed by Zhang et al. [8], who fabricated an nHA/ColI/MWCNTs composite at 0.5, 1.0, and 1.5 wt.% MWCNTs. They reported that MWCNTs can effectively stimulate the initial crystallization of HA by functioning as a specific nucleation site, with 1 wt.% MWCNTs identified as the optimal content. In a study conducted by Park et al. [9], sol-gel processing was used to fabricate MWCNTs-HA nanocomposites containing varying MWCNTs content (0.1, 0.5 and 1.0 wt.%), which were subsequently used to coat the surface of pure Ti. Their results indicated that 0.5 wt.% MWCNTs-HA coating exhibited the highest level of cytodifferentiation. Apart from that, the exceptional electrical conductivity of MWCNTs influences the pH and ion concentration in the vicinity of the material surface, promoting apatite formation by creating a favourable environment for the precipitation of apatite crystals [10]. The addition of MWCNTs can also affect the surface charge and surface free energy of the material, thereby enhancing the formation of apatite nuclei and facilitating the adsorption of ions from the physiological environment [11]. Meanwhile, Maleki-Ghaleh and Khalil-Allafi [12] investigated HA coatings incorporating 20 wt.% Ti and 1 wt.% MWCNTs fabricated using the electrophoretic deposition method. Their results showed marked improvements in hardness and adhesion strength, while biological evaluations revealed substantial enhancement in apatite formation and bone cell proliferation compared to pure HA coatings. In our previous study [13], Ti-HA composites reinforced with 5 wt.% and 10 wt.% MWCNTs were successfully fabricated using mechanical alloying and powder metallurgy methods. The effects of MWCNT content on mechanical properties were investigated, and the reduction in mechanical performance at 10 wt.% was attributed to MWCNT agglomeration, increased porosity, and poor interfacial bonding. However, the bioactivity of these composites was not investigated in the study.

The present work focuses on evaluating the bioactivity of Ti-HA-MWCNTs composites containing 5 wt.% and 10 wt.% MWCNTs to assess their potential for bone implants. By varying the MWCNTs content, the optimal MWCNTs content that maximizes the bioactivity of the Ti-HA composite can be determined, thus paving the way for the development of suitable biomaterials for orthopaedic applications.

2. MATERIALS AND METHODS

2.1 Raw materials

The starting materials included commercially pure Ti powder (cp-Ti) (Strem), HA powder (Sigma-Aldrich), and MWCNT powder (Hasrat Bestari Sdn Bhd). The cp-Ti powder had an average particle size of 139.02 μm, whereas the HA powder had a particle size of 10.36 μm. The MWCNTs, used as the reinforcing material, had a purity exceeding 95%, an outer diameter of 10 ± 1 nm, and a length range from 1 to 5 μm.

2.2 Functionalization of MWCNTs

Commercial MWCNTs are difficult to disperse in solution due to their hydrophobic surfaces, which result in poor dispersion in most solvent matrices [14]. To overcome this limitation, surface modification of MWCNTs is performed to improve dispersion and enhance suspension stability. The MWCNTs were functionalized by a strong acid solution, as recommended by Zaine et al. [15]. The acid solution was composed of sulfuric acid (H_2SO_4) and nitric acid (HNO_3) in a 3:1 ratio. Both acids had a purity of 95% to 98%, and all chemicals were supplied by Merck. The CNT surface functionalization provided by acidic treatments imparts a negative surface charge, resulting from the presence of OH, COO, and COOH groups [16]. This mixture was heated for 6 h at 100 °C. Following that, the mixture was diluted with distilled water and washed thoroughly until pH 7. The resulting solution was then filtered using membrane filters. After filtration, the MWCNTs were dried for 24 h at 80 °C to remove any volatile solvents.

2.3 Preparation of Ti-HA-MWCNTs composite

The starting materials for this study were Ti, HA, and MWCNTs powders. Ti-MWCNTs-HA with different MWCNTs content was investigated, and the amount of HA was fixed at 10 wt.%, as shown in Table 1. The powder was measured using an analytical balance with a 0.0001 g resolution and blended in a Fritsch Pulverisette 5 planetary ball mill. The milling medium was a stainless-steel jar containing steel balls at a 10:1 ball-to-powder ratio (BPR). The milling was conducted at 200 rpm for 2 h in an argon atmosphere. The as-milled particles had irregular shapes, which are generally considered to be the best forms for the press and sinter approach [17], as shown in Fig. 1. The Ti, HA, and MWCNTs powder mixtures were then compressed into steel dies using a pressing machine (Model: Specac) at a pressure of 500 MPa for 2 mins, resulting in 10 mm diameter pellets. The samples were then removed from the die, resulting in the formation of the green part. The samples were then heated in a Lenton tube furnace under an argon atmosphere. The green compacts were heated in the furnace for 2 h at a rate of 10 °C/min. After the sintering process, the samples were allowed to cool within the furnace.

Table 1. Composition of biocomposite

Composite identification	Composite composition (wt.%)			
	Composite	Titanium (Ti)	Multi-walled carbon nanotubes (MWCNTs)	Hydroxyapatite (HA)
5 wt.% MWCNTs	85Ti-5MWCNTs-10HA	0.85	0.05	0.10
10 wt.% MWCNTs	80Ti-10MWCNTs-10HA	0.80	0.10	0.10

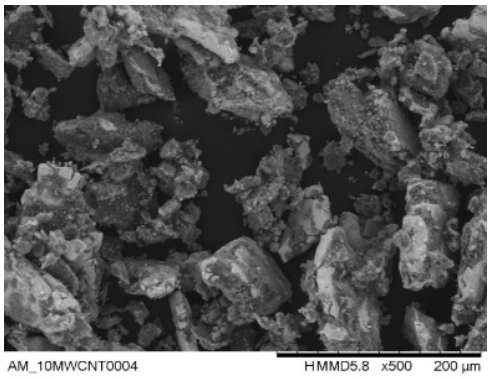


Fig. 1. SEM micrograph of as-milled Ti-HA-MWCNTs at magnification of 500x

2.4 Materials characterization

The ability of apatite formation was investigated in Hanks' Balanced Salt Solution (HBSS). The radius and thickness of samples containing 5 wt.% MWCNTs composite and 10 wt.% MWCNTs composites were measured prior to immersion in HBSS solution. By recording the sample's thickness and radius, the area can be calculated using Eq. (1), and the amount of HBSS solution can be calculated using Eq. (2). After placing the sample in the tube (Fig. 2), the calculated volume of HBSS solution was gradually poured-into the Falcon tube. The sample was completely submerged horizontally at the bottom of the tube. Consequently, the ion concentrations of HBSS solution are listed in Table 2. The sample was immersed in HBSS solution for seven days at 37 °C.

$$S_a = 2\pi r^2 + 2\pi r\mathrm{h} \tag{1}$$

where :

S_a = Surface area of cylindrical pellet (sample)

r = Radius

h = Height

$$V_s = S_a/10 \tag{2}$$

where :

V_s = Volume of HBSS solution

S_a = Surface area of cylindrical pellet (sample)

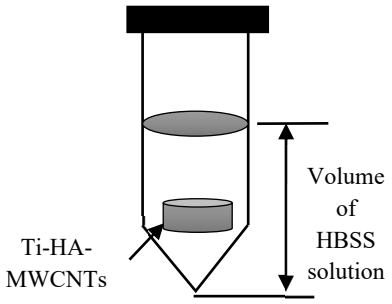


Fig. 2. Schematic diagram of Ti-HA-MWCNTs composite being soaked in HBSS solution for seven days

Table 2. The ion concentration of HBSS solution

Ion	Concentration (10^{-3})
Na^+	142.1
K^+	5.3
Mg^{2+}	0.9
Ca^{2+}	1.26
Cl^-	146.8
HCO_3^{2-}	4.2
HPO_4^{2-}	0.78
SO_4^{2-}	0.41

The weight gain of the samples resulting from apatite formation was measured after seven days of immersion in HBSS solution. Four replicate samples were used for each parameter and averaged. The weight of the sample was measured using an automatic balance before and after immersion and drying. The weight gain was calculated using Eq. (3) where W_I denotes the sample weight prior to immersion while W_F denotes the dried sample weight after immersion. To observe the surface morphology, the scanning electron microscope (SEM) Tabletop TM3030 Plus was used.

$$Weight\ gain = \frac{W_f - W_i}{W_f}\ x\ 100\% \quad (3)$$

where :

W_F = Final dry weight after 7-days immersion in HBSS solution

W_I = Initial dry weight of the sample before immersion in HBSS solution

3. RESULT AND DISCUSSION

3.1 Weight measurement

As shown in Fig. 3, the weight gain results clearly demonstrate the positive impact of incorporating MWCNTs on the bioactivity of Ti-HA composites. The 10 wt.% MWCNTs composites had the highest weight gain (1.72%), followed by the 5 wt.% MWCNTs composite (1.56%). Apart from that, a composite with 10 wt.% MWCNTs yielded the densest apatite, amounting to 0.03 g, as shown in Table 3. However, a composite containing 5 wt.% MWCNTs produced 0.02 g of apatite after drying. Several factors can explain the observed increase in weight gain and apatite formation as MWCNT content increases. First of all, MWCNTs have a higher surface area-to-volume ratio, which means there are more nucleation sites for apatite crystal formation. This facilitates the adsorption of calcium and phosphate ions from the HBSS solution, leading to the precipitation and growth of apatite on the composite surface. Secondly, the acid treatment of MWCNTs introduces functional groups, such as carboxyl (-COOH) and hydroxyl (-OH) groups, onto their surface. These functional groups interact with calcium and phosphate ions in the HBSS solution, which enhances the compatibility between the composite and the surrounding tissues, creating a more favourable environment for apatite formation. This is supported by the observation that the 10 wt.% MWCNTs composite exhibited greater precipitation of white apatite coverage compared to the 5 wt.% MWCNTs composites, as discussed in the morphological section. The denser apatite layer not only contributes to the increased weight gain but also signifies a stronger bond between the composite and the surrounding biological environment, which is crucial for implant longevity. Therefore, 10 wt.% MWCNTs composite emerges as a promising candidate for bone implant applications due to its superior bioactivity and potential for enhanced osseointegration.

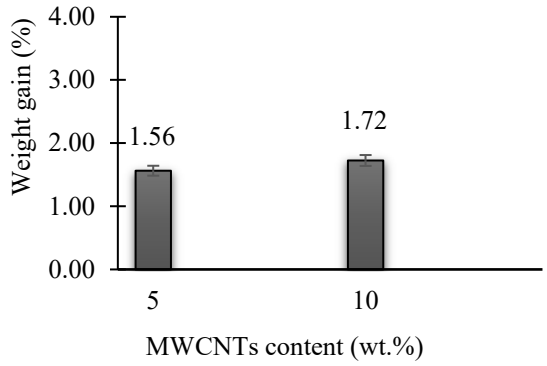


Fig. 3. Weight gain in relation to MWCNTs content

Table 3. Measurement of apatite's weight on Ti-HA-MWCNTs composite with varying MWCNTs content

MWCNTs content (wt.%)	Before immersion in HBSS solution (g)	After immersion in HBSS solution (g)	Apatite weight (g)
5	0.63	0.65	0.02
10	0.55	0.58	0.03

3.2 Morphological studies

Prior to immersion in the HBSS solution, the surface morphologies of Ti-HA-MWCNTs composite were examined to observe and verify bone-like apatite precipitation, as shown in Fig. 4. The addition of MWCNTs content creates a rougher and more textured surface on the composite due to the inherent morphology of MWCNTs, which are long and thin tubes. When incorporated into the composite, they cause the Ti-HA matrix's smooth surface to be disturbed. This is in line with Maziukiewicz et al. [18], who found that adding MWCNTs increases the composite's surface roughness. Surprisingly, the roughened surface created by MWCNTs provides favourable conditions for apatite formation, leading to increased apatite deposition on the composite surface. This is because a rougher surface has a larger surface area than a smooth one, meaning there are more sites available for apatite nucleation and growth. Moreover, the rough surface provides crevices and irregularities where apatite crystals can anchor and grow, resulting in a stronger bond between the composite and the newly formed apatite [19]. A rough surface is often more hydrophilic (water-loving), which can facilitate the adsorption of ions from the surrounding fluid, a crucial step in apatite formation [20]. Creating a white coating on a composite surface requires the formation of a calcium-containing apatite layer. As a result, increasing the content of MWCNTs (Fig. 4a) results in a more textured surface with increased surface area, which aids in the adsorption and nucleation of apatite ions in a physiological environment. Following immersion in HBSS solution (Fig. 4b), the surface appearance changed dramatically from grey to white due to the formation of phosphate precipitates, which are known to promote bone formation [21]. At 10 wt.% MWCNTs, there is a significant accumulation of apatite on the composite surface, implying that the MWCNTs act as effective nucleation sites, allowing for the growth of a continuous and well-adhered apatite layer. This is in contrast to the 5 wt.% MWCNTs composites, which contained very little apatite and had less uniform coverage. Hence, the formation of a dense and uniform apatite layer is crucial for achieving strong bonding between the implant and bone tissue, a key factor in the long-term success of orthopaedic implants. In addition, the observed morphology aligns with the weight gain results, further confirming the superior bioactivity of the 10 wt.% MWCNTs composites.

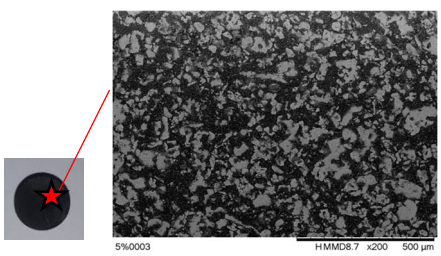
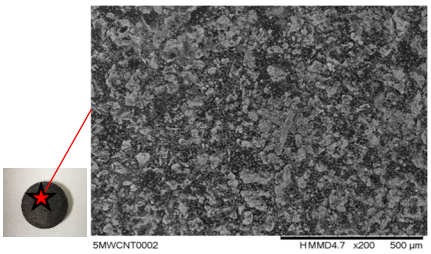
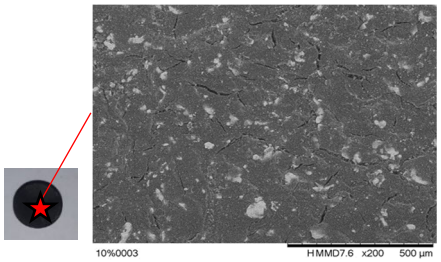
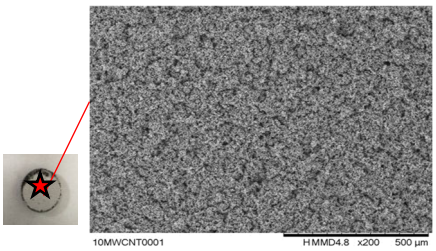
MWCNTs content (wt.%)	Before immersion in HBSS solution	After immersion in HBSS solution
5		
10		

Fig. 4. Macroscopic images and SEM micrographs (200x) of Ti-HA-MWCNTs composites with varying MWCNTs content, before and after immersion in HBSS solution for seven days

4. CONCLUSION

The study's conclusions are as follows.

i. The addition of MWCNTs into Ti-HA composites increases their bioactivity by increasing the surface area for apatite nucleation and promoting ion adsorption.

ii. The weight gain percentage and apatite formation on the composite surface increase as the MWCNTs content increases.

iii. The optimal MWCNTs content for maximizing the bioactivity in Ti-HA composites is 10 wt.%.

5. ETHICS STATEMENT

The authors declare that this research did not involve human or animal subjects. All experimental procedures were performed following the institutional Safety, Health, and Environmental (HSE) protocols of Universiti Teknologi MARA.

6. ACKNOWLEDGEMENTS/FUNDING

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7. CONFLICT OF INTEREST STATEMENT

The authors agree that this research was conducted in the absence of any self-benefits, commercial or financial conflicts, and declare the absence of conflicting interests with the funders.

8. AUTHORS' CONTRIBUTIONS

Nor Shamimi Shaari: Conceptualization, formal analysis, supervision, writing-original draft; **Shakir Zufayri Shahrum Zamir:** Methodology, formal analysis, writing-original draft; **Marina Mokhtar:** Conceptualization, methodology, validation; **Fatin Hazwani Mohamad Azahar:** Methodology, validation; **Fazira Mohamed Fadzil:** Resources, **Farrahnoor Ahmad:** Conceptualization, supervision, editing, and validation.

9. DECLARATION OF GENERATIVE AI IN THE WRITING PROCESS

During the preparation of this work, the authors used QuillBot to improve the readability and language of an early draft. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

10. DATA AVAILABILITY/SUPPLEMENTARY MATERIALS

Data sharing is not applicable to this article as no new datasets were generated or analysed during the current study.

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