Honeycomb Structures of TiO₂-modified Hydroxyapatite Composite for Microbial Filtration Application

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Abstract

Honeycomb structures of TiO₂-modified hydroxyapatite composite (HA/TiO₂) were fabricated using extrusion technique. The mixture formulations of HA/TiO₂ extrusion pastes (S1-S4) were investigated to optimize the process. The filtering effectiveness of the HA/TiO₂ honeycombs was evaluated by photocatalytic activity measurement and a bactericidal test. The effects of sintering temperature and honeycomb thickness on photocatalytic and antibacterial activities were additionally studied. The S3 and S4 honeycomb samples, sintered at 650°C, exhibited a similar trend in reducing methylene blue concentration. At 800°C, the S3 honeycomb sample showed a slightly faster reduction in methylene blue concentration compared to the S4 honeycomb. Honeycomb samples of 2 and 5 cm in thickness showed significantly greater photocatalytic activity than that of 1 cm such that methylene blue concentrations rapidly decreased after UV exposure for 24 hours. The S3 and S4 honeycomb samples also exhibited decomposition of both gram-negative E. coli and gram-positive S. aureus. Approximately 50% of gram-positive S. aureus and gram-negative E. coli were decomposed by the honeycombs in the sample-thickness dependent manner after 2 hours of UV exposure. Low survival ratios of bacteria (5% - 10%) were observed after 6 hours of UV exposure and the 2 mm and 5 mm thick honeycomb samples showed the greatest and most similar antibacterial activities.

Keywords: Honeycomb; Hydroxyapatite; Titanium dioxide; Antibacterial; Photocatalytic activity

Introduction

Hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂), a calcium phosphate based compound which closely resembles natural bones and teeth, has been extensively used for biomedical applications, particularly for replacing human hard tissue [1-6]. It possesses excellent osteoconductivity, bioactivity, ionic exchange property and high stability under reducing and oxidizing situations leading to the adsorption ability for microbial bacteria and viruses [7-10]. HA is capable of adsorbing molecules on its surface but not decomposing molecules nor making microbes harmless, therefore, saturation will be reached over time [11].

Nanoparticle titanium dioxide (TiO₂) is a basic material extensively utilized in daily life, for example, as a pigment for paints, cosmetics and foodstuffs because of its effective performance as a photocatalyst. It is of great interest due to its potential in the oxidative decomposition of organic compounds, strong oxidizing power under UV, chemical stability, non-toxicity and long-term stability against photo and chemical corrosion [7-12]. However, TiO₂ can decompose only substances that come into direct contact, and the decomposition requires the presence of light, especially in the ultraviolet range [13-15]. By modifying the HA with TiO₂, their advantages can be combined-the HA adsorbs molecules that will then later be decomposed by TiO₂. The HA/TiO₂ composite has, therefore, the potential to be used for solving environmental problems such as water and air pollution due to its photocatalytic, antibacterial and antivirus properties. Nishikawa et al. reported that HA/TiO₂ composite exhibited higher photocatalytic activity than commercial TiO₂ and showed the rapid and complete oxidative decomposition of acetaldehyde [16]. The use of HA/TiO₂ composite as a bone scaffold coupled with collagen type I in the form of nanofiber mats fabricated by the electro spinning technique has also been reported [17]. Coating of hydroxyapatite on titania has also been reported for bone substitute application [18]. Moreover, the composite of HA/TiO₂ can be applied in the field of sonocatalysis to damage bovine serum albumin (BSA) under ultrasonic irradiation [19].

At present, for the filtration application, most research has concentrated on coating the thin film of HA/TiO₂ composite onto substrate materials. However, the photocatalytic activities of the HA/TiO₂ particles coated on the substrate decreased compared to those in powder form, due to a decrease in the surface area of the particles. In addition, coating the HA/TiO₂ composite onto fibrous substrates for microbial filter application was also reported to have limitations in use. In the coating, particles can either deposit onto the fibers’ surfaces or penetrate into the inter-fiber space which may not be fully exposed to UV light. The latter could lead to a decrease in photocatalytic activity [20]. By forming HA/TiO₂ composites into porous structures, which provide higher surface area and increase exposure area to UV light, it is possible to increase photocatalytic efficiency. Honeycomb is a complex structure composed of several small unit cells, which have a thin wall between each cell. Honeycomb ceramics are widely used as a catalytic converter in automobile gas exhausts and a catalyst carrier [21]. Ceramic honeycomb structures provide the high surface areas with least volume. The geometric surface area of the carrier due to the characteristics of the porous thin wall greatly increased and improved thermal shock resistance [22,23]. Industrially, extrusion is one of the most popular techniques with which to fabricate honeycomb ceramics. Many factors influencing the properties of the final honeycomb products, particularly the compositions of raw materials, need to be controlled to achieve the desirable products.
In this study, extrusion technique was employed to fabricate HA/TiO₂ composite at a ratio between HA to TiO₂ of 50:50 by weight. Four different mixture formulations of extrusion pastes were investigated in order to fabricate HA/TiO₂ honeycombs. The effects of sintering profiles and honeycomb thickness on photocatalytic activity and bactericidal performance were investigated and discussed in terms of their potential uses as microbial filters.

Experimental Procedure

Preparation and characterization of pure hydroxyapatite (HA)

Pure hydroxyapatite was prepared using a solid-state reaction method. Mixture powders of calcium carbonate (CaCO₃) and calcium phosphate dibasic (CaHPO₄) reacted as shown in equation (1).

\[ 4\text{CaCO}_3 + 6\text{CaHPO}_4 \rightarrow \text{Ca}_10(\text{PO}_4)_6(\text{OH})_2 + 2\text{H}_2\text{O} + 4\text{CO}_2 \] (1)

All raw materials were used as received. CaCO₃ (Carlo Erba Reagent, Italy) and CaHPO₄ (Sigma-Aldrich, Germany) were ball-milled with zirconia balls in the presence of deionized water as a medium for 48 hours, before drying. The mixture was then calcined at 900°C for 2 hours to produce pure hydroxyapatite powder. The obtained powders were ground and sieved to attain a particle size of less than 10 µm. Characterization of HA powders after calcination was done using x-ray diffraction: XRD (JEOL JDX 3530) with CuKα source (Kₐ = 1.5406 nm) operating at 30 mA and 30 kV. XRD measurement was conducted at 20-60° 2θ, scan speed of 2°/min with a step size of 0.02°. The XRD spectra were analyzed using Jade software and ICDD cards. Particle size analysis was measured by laser diffraction technique (Mastersizer 2000, Malvern instruments, UK).

Preparation and characterization of HA/TiO₂ composite

Anatase phase TiO₂ (Sigma-Aldrich, Germany) was used as received. HA/TiO₂ composite was prepared by dry ball milling the mixture between HA and TiO₂ powders at the composition ratio of 50:50 (HA: TiO₂ 50:50), using ZrO₂ balls for 3 hours. The obtained HA/TiO₂ composite was then calcined at 650°C for 2 hours. The composite was then wet milled for another 24 hours using deionized water as the medium under ZrO₂ balls to achieve a particle size of less than 5 micron, followed by drying at 100°C. The particle size of HA/TiO₂ composite was analyzed by laser diffraction technique (Mastersizer 2000, Malvern instruments, UK). Analysis of their phases was carried out using XRD: JEOL JDX 3530.

Preparation and characterization of honeycomb structure

Four different mixture formulations of the extrusion pastes (S1-S4) as summarized in Table 1 were prepared for fabricating the HA/TiO₂ honeycombs. The paste compositions consisted of HA/TiO₂ composite powder as the main component, processing additives which were water as medium, methylcellulose (MC) and starch as binders, polyethylene glycol (PEG) and flour as plasticizers, sodium carbonate as a dispersant as well as some lubricants such as oil. Each formulation was uniformly mixed using a high speed mixer before being further transferred to a 3-roll mill for homogenous mixing. These paste batches were then de-aired and extruded using a screw extruder (FM-30, Miyazaki Iron Work, Japan) into cylindrical honeycomb structures of 50 mm in diameter with a cell density of 72 cell/inch² and having three different thicknesses (1, 2 and 5 cm). The extruded HA/TiO₂ honeycomb samples were covered with semi-dry cloth to let them slowly dry at room temperature. After drying, the honeycomb samples of selected formulations, S3 and S4, were sintered at 400°C for 2 hours and then ramped up to three different temperatures (650, 800 and 1000°C) held for 4 hours, followed by furnace cooling to room temperature at a rate of 50°C/h. Phases and microstructures of the obtained HA/TiO₂ honeycombs were analyzed using XRD: JEOL JDX 3530 and scanning electron microscopy (SEM: JEOL JSM-5410).

Physical and mechanical properties

Five specimens each of S3 and S4 honeycomb structures, sintered at 650°C and 800°C, were used to measure bulk density and total porosity based on Archimedes’ principle. The honeycombs were dried in an oven at 100°C and cooled to room temperature before testing. In accordance with Archimedes’ principle, a sample was completely dried and then weighed. The weight was recorded as W_w. After weighing, the sample was placed in a beaker with the deionized (DI) water to allow the specimen to be soaked in the water for 24 hours. Within 24 hours, the specimen was assumedly saturated and then a vacuum was applied to force the water into the open pores of the honeycomb structures until no air bubbles emerged from the structure. After that, the beaker was placed on a stand over a balance. A density kit was hung below the water level but not touching any sides of the beaker in a position ready to determine the density of the sample. The sample was lifted onto the wire and the suspended weight was recorded as W_s. Finally, the sample was removed from the water, rapidly blotted, and weighed to attain the saturated weight, W_s. The bulk density of the sample, D_b, was calculated as

\[ D_b = \frac{W_s \times \rho_w}{W_w - W_s} \]

where \( \rho_w = 1 \text{ g/cm}^3 \) at 25°C

The total porosity of sample, P, was evaluated as

\[ P = \frac{D_t - D_b}{D_t} \]

where \( D_t \) is the theoretical density of HA/TiO₂ (50:50) composite (3.468 g/cm³)

For mechanical properties testing, the specimen was prepared by extruding HA/TiO₂ composites into bars (6 mm wide x 5 mm long) and sintered at 650°C and 800°C. The 3-point bending strength of the sintered specimen was tested using a universal testing machine (Instron 55R4502, USA) with 10kN load cells. The crosshead speed was set at 0.5 mm/min and the span length to 20 mm. Five specimens were tested to obtain average values and standard deviations.

Investigation of photocatalytic activity

The photocatalytic activities of HA/TiO₂ honeycomb structures were investigated by measuring the degradation of methylene blue (MB) under UV irradiation. For comparison, MB solution without honeycomb sample was used as a blank. The evaluation was conducted

<table>
<thead>
<tr>
<th>Sample</th>
<th>HA/TiO₂ (wt%)</th>
<th>DI water</th>
<th>MC (wt%)</th>
<th>PEG</th>
<th>Na₂CO₃</th>
<th>Oil</th>
<th>Starch</th>
<th>Flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1</td>
<td>63.45</td>
<td>22.46</td>
<td>5.08</td>
<td>2.54</td>
<td>0.76</td>
<td>5.71</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S2</td>
<td>59.95</td>
<td>21.58</td>
<td>9.59</td>
<td>0.36</td>
<td>0.24</td>
<td>5.28</td>
<td>2.99</td>
<td>-</td>
</tr>
<tr>
<td>S3</td>
<td>57.47</td>
<td>22.99</td>
<td>10.34</td>
<td>0.92</td>
<td>0.23</td>
<td>5.17</td>
<td>2.87</td>
<td>-</td>
</tr>
<tr>
<td>S4</td>
<td>55.07</td>
<td>25.88</td>
<td>8.81</td>
<td>0.66</td>
<td>0.22</td>
<td>4.96</td>
<td>4.40</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 1: Formulations of extrusion paste studied in this work (%wt).
in a closed chamber equipped with UV-A lamps (315-380 nm). 250 mL of 5 ppm MB solution was added into a beaker, and the sample was put into the prepared MB solution. The beaker was placed into chamber and dark closed (no UV light) for 1 hour for initialization. Then, the MB solution was sampled out and measured for UV absorption (600-700 nm) on a UV spectrophotometer (JASCO-V530) and the absorption at peak (665 nm) was used to determine MB concentration at initial time (t0). UV lamps in the chamber were then turned on to allow photocatalytic activity. After 1 hour, the lamps were turned off and MB concentration at the testing time of 1 hour (t1) was determined. The same procedure was continued for other exposure times (1, 3, 6, and 24 hours) to determine the corresponding MB concentrations at t1, t3, t6, and t24.

**Antibacterial Activity Test**

Two types of bacteria, *E. coli* ATCC 8739, a Gram-negative bacterium, and *S. aureus* ATCC 6538, a Gram-positive bacterium, were chosen as model microorganisms in this study. Both bacteria, stored in glycerol at -80°C, were plated on nutrient agar, a mixture of nutrient broth (NB) and Bacto agar (Difco), cultured overnight at 37°C for 24 hours and stored at 4°C. Prior to each experiment, the strains were inoculated in sterile test tubes in NB and cultured at 37°C for 12 hours in order to reach the stationary growth phase. The cultures were harvested by centrifugation and resuspended in phosphate buffered saline (PBS, pH 7.4) to OD600 of 0.4, corresponding to an approximate concentration of 10^8 colony-forming units (cfu)/mL.

Honeycomb specimens were autoclaved and kept separate until use in a sterile beaker. The bacterial suspension (40, 60 and 100 mL) was added to the honeycomb samples sized 1, 2 and 5 cm, respectively and in a sterile beaker. The bacterial suspension (40, 60 and 100 mL) was enumerated using the spreading plate method on nutrient agar (NA) after a series of dilutions of the sample in normal saline.

As expected, for HA/TiO2 honeycomb sintered at 800°C, few small peaks of β-TCP due to the decomposition of HA into β-TCP as well as the peaks of the anatase phase of TiO2 were observed. Generally, HA can decompose into β-TCP at a sintering temperature range of 800-1350°C [2,24,25]. With increasing sintering temperature, HA tended to completely decompose into β-TCP coupled with more transformation of anatase to rutile of TiO2. The peaks related to CaTiO3 were also observed due to the reaction between TiO2 and Ca2+ in the hydroxyapatite site, particularly at a sintering temperature of 1000°C. As a photocatalyst, anatase TiO2 exhibited higher photocatalytic activity than other phases of TiO2 (rutile and brookite) [7-12]. The high intensity of peaks relating to the anatase TiO2 observed in the prepared HA/TiO2 composite powder implied superior crystalline size and high crystallinity. The optimal sintering temperature range of 600°C to 950°C for TiO2 had been reported to avoid the transition of anatase to rutile.
rutile [26-28]. Therefore, the sintering temperatures 650°C and 800°C were selected for further investigation in this study.

The influence of sintering temperature on the morphology of HA/TiO₂ honeycombs was investigated. In this step, S3 and S4 formulations, which yielded complete shapes of honeycomb structure, were employed. Figure 3 illustrates the morphology of the honeycomb structure. Overall, irregular shapes of HA and TiO₂ crystals were observed in which small crystals of anatase TiO₂ were distributed and embedded in larger HA crystals with micropore sizes in the range of 0.23-0.28 µm. Similar morphologies were observed in honeycombs sintered at 650 and 800°C. Meanwhile, honeycomb samples sintered at 1000°C exhibited larger pore sizes (~0.40 µm) compared to those sintered at 650°C and 800°C. HA/TiO₂ grain size increased with sintering temperature, thus resulting in the decrease in porosity.

From the results shown earlier (Figure 2), honeycombs sintered at 1000°C exhibited transformation from anatase to rutile TiO₂ whereas those sintered at 650°C and 800°C did not. Therefore, the honeycombs sintered at 650°C and 800°C were chosen for further investigation on the properties of HA/TiO₂ honeycombs such as strength, photocatalytic activity, and anti-bacterial performance.

Table 2 presents the bulk density and total porosity of the S3 and S4 honeycomb structures of different sintering temperature and thickness. A small discrepancy in density (~30%) and porosity (~60%) was observed among S3 and S4 samples sintered at 650°C and 800°C. It seems that samples sintered at 800°C exhibited a slightly higher density, particularly in the thick samples (5 cm). It might be difficult to clearly determine the density and porosity of the honeycomb structure using Archimedes’ principle, compared to SEM images, due to the limitations of the technique and the complexity of the honeycomb structure itself. Therefore, neither a trend of increasing nor decreasing for both density and porosity could be clearly identified with sintering temperature.

The bending strength of honeycomb structures sintered at 650°C and 800°C are reported in Table 3. The honeycombs sintered at 800°C possessed a greater bending strength than those sintered at 650°C due to higher densification and less porosity, in micro scale, as evidenced by the SEM images (Figure 3). This implies that the strength of the honeycomb could be improved by increasing the sintering temperature. At a higher sintering temperature (1000°C), however, the phase transformation from anatase to rutile occurred and could reduce the desired photocatalytic activity.

### Table 2: Density and porosity of honeycomb samples.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Temperature (°C)</th>
<th>Density (% ± SD.</th>
<th>Porosity (% ± SD.)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Thickness (cm) 1</td>
<td>2</td>
<td>5</td>
</tr>
<tr>
<td>S3</td>
<td>800</td>
<td>33.97 ± 1.26</td>
<td>34.23 ± 1.34</td>
</tr>
<tr>
<td>S4</td>
<td>650</td>
<td>32.62 ± 0.78</td>
<td>35.30 ± 1.17</td>
</tr>
<tr>
<td></td>
<td>800</td>
<td>33.44 ± 1.41</td>
<td>31.81 ± 0.90</td>
</tr>
</tbody>
</table>

The photocatalytic activities of S3 and S4 HA/TiO₂ honeycombs of thickness 1, 2, and 5 cm sintered at 650°C and 800°C were evaluated using 5 ppm MB solution. According to the results, the
TiO2 peaks observed in the HA/TiO2 honeycomb sintered at 800°C at 650°C. This may be related to the higher intensities of anatase showing slightly higher rates of MB decomposition than those sintered at 650°C. This may be related to the higher intensities of anatase TiO2 peaks observed in the HA/TiO2 honeycomb sintered at 800°C (Figure 2e). The difference, however, was not significant. The S3 and S4 honeycombs exhibited comparable rates of MB decomposition. At the same sintering temperature, HA/TiO2 honeycombs of thickness 2 and 5 cm exhibited comparable rates of MB decomposition, but slightly greater than that of the 1-cm thick honeycomb (p<0.07). The lower initial MB concentration (at 0 hr) of 5-cm thick honeycomb, compared to those of the 1 and 2 cm thick samples, was thought to be due to its greater area for MB adsorption during initialization. After 24 hours of UV exposure, the MB solutions in the presence of the HA/TiO2 honeycombs were effectively decomposed as the solution became clear.

### MB concentration decrease with UV exposure time in the presence of HA/TiO2 honeycombs and the rate of MB decomposition was rapid during the first 3 hours, whereas the blank (without HA/TiO2 sample) showed no significant change in concentration (Figures 4a and 4b). Overall, HA/TiO2 honeycombs sintered at 800°C tended to show slightly higher rates of MB decomposition than those sintered at 650°C. This may be related to the higher intensities of anatase TiO2 peaks observed in the HA/TiO2 honeycomb sintered at 800°C (Figure 2e). The difference, however, was not significant. The S3 and S4 honeycombs exhibited comparable rates of MB decomposition. At the same sintering temperature, HA/TiO2 honeycombs of thickness 2 and 5 cm exhibited comparable rates of MB decomposition, but slightly greater than that of the 1-cm thick honeycomb (p<0.07). The lower initial MB concentration (at 0 hr) of 5-cm thick honeycomb, compared to those of the 1 and 2 cm thick samples, was thought to be due to its greater area for MB adsorption during initialization. After 24 hours of UV exposure, the MB solutions in the presence of the HA/TiO2 honeycombs were effectively decomposed as the solution became clear.

### Antibacterial activity

A photocatalytic decomposition reaction of our HA/TiO2 has been previously reported to be applicable to deactivate microorganisms under weak UV light [20]. In this study, the in vitro antibacterial activities of the S3 and S4 HA/TiO2 honeycombs of different thicknesses under room ambience with UV light were investigated. The S3 and S4 HA/TiO2 honeycombs were prepared from two similar compositions of extrusion paste that differed in the additive selection (Table 1). Figures 5a and 5b, respectively, show the curves of survival ratio (%) versus culture time for the HA/TiO2 honeycombs against gram-positive S. aureus and gram-negative E. coli. The smaller the survival ratio, the higher the antibacterial activity of the sample. The survival ratios of both bacterial strains decreased with incubation time for all tested HA/TiO2 honeycombs. The S3 and S4 honeycombs exhibited comparable antimicrobial activity as their survival curves with similar sample lengths could almost be superimposed for both S. aureus and E. coli. This may be explained by both the starch and flour respectively selected for the S3 and S4 honeycombs as processing additives being generally neither bactericidal nor bacteriostatic agents. Moreover, their other compositions were almost quantitatively and qualitatively similar.

At the UV exposure time of 2 hours, the survival ratios of both bacterial strains found for the S3 and S4 honeycombs significantly decreased as the sample length increased (p<0.003). The explanation for this is that the longer the sample, the more the HA/TiO2 photocatalyst is incorporated into the sample and the faster its bactericidal activity is initiated. At longer exposure times (4 and 6 hours), only 5 - 15% of bacteria remained in a proliferative state and the 1-cm thick honeycomb samples, containing the least amount of HA/TiO2, still showed the highest survival ratios, as compared to the corresponding 2 and 5 cm thick samples (p<0.003 for S. aureus and p<0.001 for E. coli). However, the bactericidal activities of the 2 and 5 cm thick samples after 4 and 6 hours exposure became statistically comparable (p<0.088 for S. aureus and p<0.700 for E. coli).

### Conclusion

The honeycomb structures of TiO2-modified hydroxyapatite (HA/TiO2) composite were successfully fabricated through the extrusion method. The complete honeycombs of 50 mm in diameter and cell density of 72 cell/inch2 were extruded in three different thicknesses (1, 2 and 5 cm). Fabrication of honeycomb structure requires a slow and consistent drying process before sintering. The appropriate sintering temperatures were 650 or 800°C. At these temperatures, the crystallizations of hydroxyapatite and anatase TiO2, which were required for better photocatalytic and antibacterial activities as well as stronger samples, were achieved.

HA/TiO2 honeycombs sintered at 800°C exhibited slightly higher photocatalytic activity, compared to those sintered at 650°C, due to the greater amount of anatase TiO2. The S3 and S4 honeycombs exhibited comparable rates in reducing methylene blue concentration. For the same sintering temperature, the 2- and 5-cm thick honeycombs showed greater photocatalytic activity than that of the 1-cm thick honeycomb.

Antibacterial activity testing results reported that 50% of bacteria were decomposed after 2 hours of testing. With increasing culture time, less numbers of bacteria could survive -around 5-10% of bacteria survived after 6 hours of culture time. The S3 honeycomb sample did not exhibit significantly higher decomposition of both gram-negative E. coli and gram-positive S. aureus compared to S4. The thickness of the honeycomb samples also influenced the antibacterial performance of the samples for both E. coli and S. aureus. It has been found that 2cm and 5cm honeycomb samples showed greater antibacterial activity than the 1cm-honeycomb through the culture period of time.
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There is no financial or other relationship that might be perceived as leading to a conflict of interest.

The authors declare that there are no conflicts of interest.

References

22. Ceramic Honeycomb Substrate
23. Ceramic honeycomb