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Analyzing antibacterial and antifungal properties of polypropylene/hydroxyapatite composites

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The purpose of this study was to confirm the antifungal and antibacterial properties of polypropylene (PP) added with different weight ratios of hydroxyapatite (HAp). Liquid PP was prepared by applying heat to solid PP. and then, HAp was added to liquid PP. According to weight ratios of HAp, three samples were prepared by the injection molding method. The phase ratio of the sintered powder was measured by a multipurpose X-ray diffractometer (MP-XRD). The XRD result indicated that the sintered powder component was 95% $Ca_5(PO_4)_3(OH)$ and 3% CaO. That result was the composition of high purity HAp. ASTM G21 test was conducted to confirm the antifungal properties of the composites. All results showed the absence of fungi on the composite surfaces. Also, JIS Z 2801 test was conducted to confirm the antibacterial properties of the composites. Bacterial growth was found on the surface of the composites containing < 5% HAp. Based on these results, we can conclude that PP/HAp composites with HAp weight ratios of 5% or higher will exhibit desired antifungal and antibacterial properties.

Key words: Polypropylene, Hydroxyapatite, Composite, Antifungal, Antibacterial.

Introduction

Antifungal and antibacterial properties had considered a valuable study at the field of real life or medicine. So, In order to suppress the growth of bacteria and fungi on the surfaces, studies have been conducted about materials with these properties [1-4].

Among of them, polypropylene (PP) had attracted attention due to its characteristic that it had low moisture absorptivity. In case of bacteria and fungi, appropriate growth was impossible under the low humidity conditions. As a result, they become to death.

To improve the antibacterial and antifungal properties of PP, the study was currently conducted by coating method with gold [5], gold-palladium [6], silver [7-10], and adding the matter which has antibacterial and antifungal properties [11, 12].

However, metal coating method caused increasing the production cost and the equally distribution technology of metal particle was required. Also, in case of the sample which added weak antimicrobial agent, antibacterial property was shown low effect to bacteria. And, if a large amount of weak antimicrobial agent was applied. It caused losing the original properties of basic material such as physical, chemical properties. And, it also caused raising the sample prices

In order to improve the antibacterial and antifungal properties, our study has been progressed by using the hydroxyapatite. HAp was already well known for representative bio-ceramics [13-15]. It had excellent biocompatibility and biocompatible. Besides, HAp can carry the positive charge and has adsorption characteristic at proteins of acidic, neutral and basic, virus and bacteria [16-20]. so, these characteristic can occur improving the antibacterial and antifungal properties.

The aim of this work was to evaluate the antibacterial and antifungal properties of PP added with different weight ratios of HAp. Each PP/HAp composites were prepared by Injection molding. Phase ratio of produced powders was investigated by multipurpose X-ray diffractometer (MP-XRD). And, Quantitative fungal and antibacterial activity of PP/HAp composite against was evaluated following the guidelines of ASTM G 21-2009 and JIS Z 2801:2010.

Experimental

Materials

The polypropylene (PP) (H551 from GS Caltex Corporation) and hydroxyapatite (HAp) were used to produce the composites. Calcium carbonate and phosphoric acid were uniformly mixed for 24 h to produce high-purity HAp with a stoichiometric mixing ratio of 1.67. After drying, the mixture was sintered for 4 h at 1350 °C with the temperature increasing and decreasing at a rate of 5 and 4 °C/min, respectively. The HAp powder obtained was crushed by ball milling and was sieved through a 325 mesh (particle diameter ≤ 45 μm). Then, the phase ratio of the sintered powder

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Table 1. Descriptions for ASTM ratings for evaluating the different degrees of fungal growth.

ASTM Rating	Observed growth on samples
0	none
1	Traces of growth (less than 10%)
2	Light growth (10-30%)
3	Medium growth (30-60%)
4	Heavy growth (60% to complete coverage)

was measured with a multipurpose X-ray diffractometer (MP-XRD) to confirm the generation of HAp.

Sample preparation

Each 6g solid PP sample (square shape; 50 × 50 × 1 mm^3 dimensions) was placed into a beaker and heated at 180 °C to produce liquid PP. Subsequently, HAp based on the PP weight of 1%, 3% and 5% was added to each liquid PP sample. Mulling was conducted to ensure suitable HAp content in the composites. Injection molding was then performed to generate test composites. Besides, when testing the ASTM G21, the samples treated with sanding process were used.

Antifungal tests

The ASTM G21-2009 was used to evaluate the antifungal properties by measuring the resistance of the synthetic polymer against fungi. The fungi used in this test were *Aspergillus niger* (ATCC 9642), *Chaetomium globosum* (ATCC 6205), *Penicillium pinophilum* (ATCC 11797), *Gliocladium virens* (ATCC 9645), and *Aureobasidium pullulans* (ATCC 15233). The fungi were grown on solid medium or spore suspension. A small volume of spore suspension was collected and sprayed directly onto the surface of test samples placed in petri dishes filled with minimal salts agar. All petri dishes were sealed to maintain sufficient humidity and incubated for up to 28 days. Subsequently, a performance score which was indicated changed performance score that was ASTM rating in Table 1 was given to each sample based on the individual fungal growth.

Antimicrobial tests

The JIS Z 2801 : 2010 was used to analyze the antibacterial properties by measuring the resistibility of the composite to bacterial growth. That test was used some bacteria, such as *Staphylcoccus aureus* (ATTC 6538) and *Escherichia coli* (ATCC 25922).

Bacterial solutions were applied onto the surfaces of the samples placed in petri dishes. Each sample was covered with a sterilization-treated film (STOMACHER 400 POLY-BAG) before the petri dish cap was put back in place. The bacteria were cultured in an incubator for 24 h under full growth conditions (35 °C ± 1 °C; humidity 90%). Then, the surface of each sample, film, and petri dish to which bacteria were attached were washed by neutralization solutions, and a number of bacteria per square centimeter were counted.

Results and Discussion

Multipurpose X-ray diffractometer

Multipurpose X-ray diffractometer (MP-XRD) analysis was performed to confirm the generation of HAp. The analyzed MP-XRD result was shown in Fig. 1. The sintered powder was contained 97% HAp and 3% CaO. Therefore, it was confirmed that the sintered powder was contained high purity of hydroxyapatite (HAp).

Antifungal tests (ASTM G 21-2009)

The composites were investigated by ASTM G 21-2009. As can be seen in Fig. 2, Blank sample was shown many fungi was grown on the surface of polypropylene (PP), but others PP added with HAp

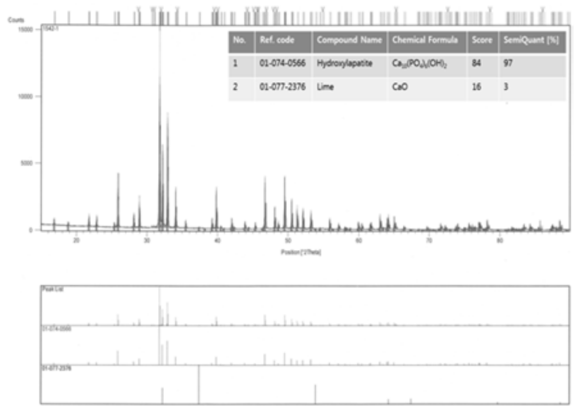

Fig. 1. XRD spectrum showing the phase ratio of the sintered powder.

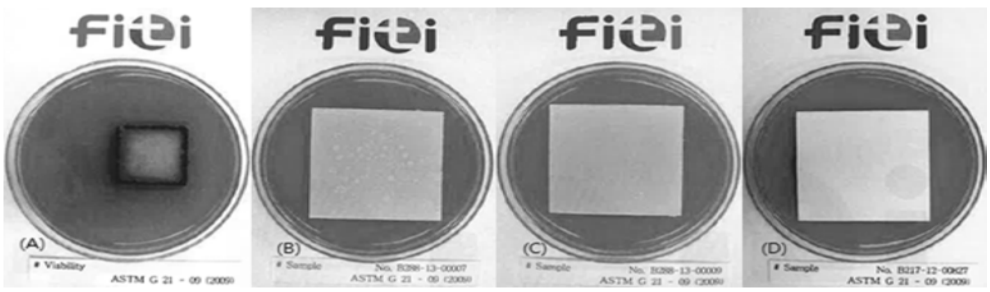

Fig. 2. Antifungal test results of (A) blank PP and PP/HAp composites containing (B) 1%, (C) 3%, or (D) 5% HAp.

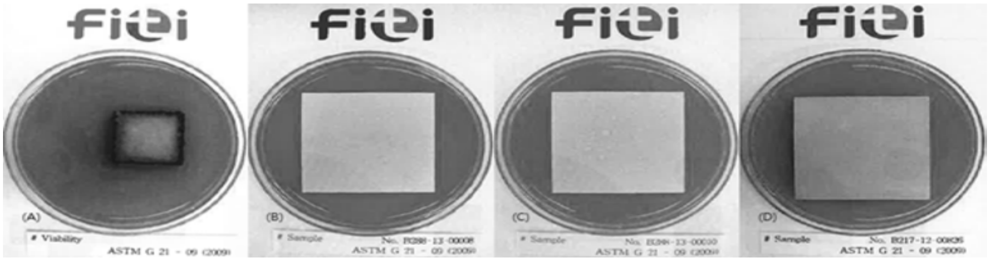

Fig. 3. Antifungal test results of (A) blank PP and PP/HAp composites containing (B) 1%, (C) 3%, or (D) 5% HAp treated by the sanding process.

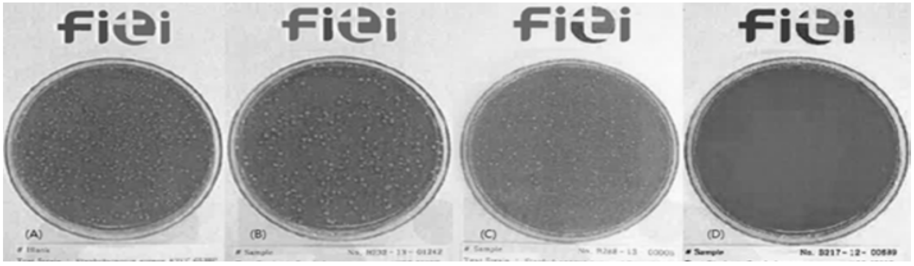

Fig. 4. Antibacterial test results of (A) blank PP and PP/HAp composites containing (B) 1%, (C) 3%, and (D) 5% HAp using *Staphylococcus aureus* (ATCC 65380).

were shown that any fungi were not grown on the surface of the PP added with HAp and ASTM rating was obtained zero degree.

This result was thought to be caused by the adsorption property of HAp located on the surface of PP [22-23]. Because of its ability to combine with fungi, HAp can be combined with fungi. As a result, fungal growth was interrupted and antifungal environment was created over the PP surface.

Furthermore, when occurring the abrasion assumed by connecting external factors. To confirm whether antibacterial property of this composite was indicated or not, samples treated with sanding process were tested.

The samples treated with the sanding process showed same results of non-treated samples for fungal growth on the polymer surfaces (Fig. 3) and ASTM rating was obtained zero degree, which can think that the distribution of HAp was equal throughout the samples.

Antibacterial tests (The JIS Z 2801 : 2010)

First, Fig. 4 showed the antibacterial test results using *Staphylococcus aureus* (ATCC 65380). *S. aureus* shows decreased viability on the samples added with HAp. Table 2 presents the accurate bacterial number in the neutralization solutions from the initial bacterial growth to full growth in 24 h. *S. aureus* growth was found on the surfaces of composites containing < 5% HAp.

Table 2. Number of *Staphylococcus aureus* (ATCC 65380) per square centimeter.

Test item		Test results	
		blank	HA
Sample A (add 1% HAp)	initial bacterial content	1.9×10^4	1.9×10^4
	24 hours after bacterial reduction	1.4×10^4	6.2×10^3
Sample B (add 3% HAp)	initial bacterial content	1.3×10^4	1.3×10^4
	24 hours after bacterial reduction	1.9×10^5	8.1×10^3
Sample C (add 5% HAp)	initial bacterial content	1.8×10^4	1.8×10^4
	24 hours after bacterial reduction	1.5×10^4	< 0.63

In contrast, in the case of recruitment stock of HAp

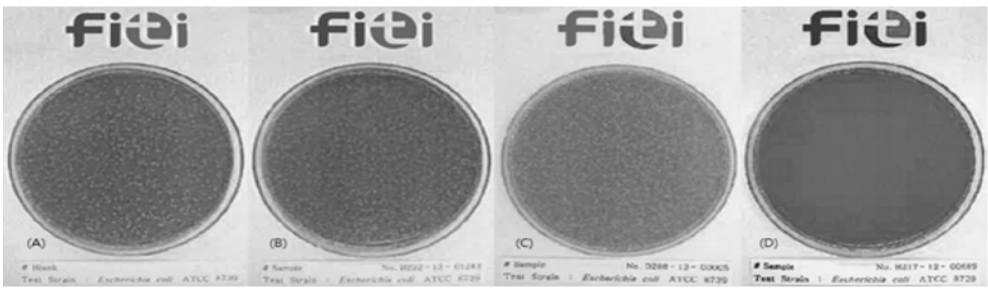

Fig. 5. Antibacterial test results of (A) blank PP and PP/HAp composites containing (B) 1%, (C) 3%, and (D) 5% HAp using *Escherichia coli* (ATCC 25922).

Table 3. Number of *Escherichia coli* (ATCC 25922) per square centimeter.

Test item		Test results	
		blank	HA
Sample A (add 1% HAp)	initial bacterial content	1.4×10^4	1.4×10^4
	24 hours after bacterial reduction	8.8×10^5	1.2×10^4
Sample B (add 3% HAp)	initial bacterial content	1.2×10^4	1.2×10^4
	24 hours after bacterial reduction	1.0×10^5	2.0×10^3
Sample C (add 5% HAp)	initial bacterial content	1.3×10^4	1.3×10^4
	24 hours after bacterial reduction	1.3×10^6	< 0.63

reached at 5%, the growth of *S. aureus* was dramatically restricted. And, as can be seen from the Table 2, the initial dose was 1.3×10^4 per square centimeter, after 24 h, the amount of the final survival was almost zero point per square centimeter.

Second, Fig. 5 and Table 3 present the results of antibacterial tests using *E. coli* (ATCC 25922). Similar results were obtained as compared to those with the antibacterial tests using *S. aureus*. The growth of *E. coli* was detected on the surfaces of composites with recruitment stock of HAp < 5%

In contrast, in the case of recruitment stock of HAp reached at 5%, the growth of *E. coli* was restricted, and the bacterial count was approximately zero point per square centimeter. After 24 hours.

The results were thought to be related to the properties of HAp. [20] The absorption ability was regarded as the most relevant one. [21-23] HAp was a type of ampholite with both positive and negative charges in solution. Therefore, HAp exhibited good absorption ability due to distinctive surface charges. In addition, HAp showed characteristic alkaline properties, which can create a protective environment for PP against the growth of bacteria.

Conclusions

The propylene (PP)/hydroxyapatite (HAp) composites were produced with adding HAp to liquefied PP at different weight ratios by conducting injection molding method. The phase ratio of the sintered powder was confirmed by MP-XRD. The antifungal and antibacterial properties of the samples were measured by the ASTM G21 and JIS Z 2801 methods, respectively. The ASTM G21 test indicated that, after 28 days of culture, fungi did not grow on the surface of any composites. The JIS Z 2801 results showed that no bacteria were present on the surface of specimens, when the addition of HAp was reached 5%.

Based on these results, we may conclude that the addition of HAp was required 5% or higher of the PP weight to obtain the desired antifungal and antibacterial properties in PP/HAp composites. PP with added HAp will show good potential in gaining broader applications in a variety of research and industrial realms in the future.

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