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Hydroxyapatite-coated antibacterial air filters

Seung-Hyun Lee[a], Su-Chak Ryu[a,*] and Hong Chae Park[b]
[a]*Department of Nanomedical Engineering, Pusan National University, Miryang 627-706, South Korea*
[b]*Department of Materials Science and Engineering, Pusan National University, Pusan 609-735, Korea*

The aim of this study was the filtration of microbes in air using a hydroxyapatite (HAp)-coated filter. The HAp powder was synthesised using $CaCO_3$ and H_3PO_4 at 1350 °C. The microstructure of the HAp-coated filter prepared via a spraying method was evaluated by scanning electron microscopy (SEM), and it was found that the HAp particles on the surface of the filter formed a compact coating. We tested the apparent density and tensile strength of the coated filter according to the requirements of the Korean Standards Association. The apparent density of the coated filter was 369.25 g cm^{-3}. Tensile strength of length and width section was 280 N and 270 N, respectively. The absorbability of the coated filter was tested to investigate whether HAp was capable of removing bacteria and viruses. A solution of 1% HAp particles displayed 90-99% absorbability. Finally, the antibacterial activity was evaluated, which demonstrated that the coated filter completely removed E. coli.

Key words: Hydroxyapatite, Disc diffusion assay, Clear zone, Absorbability, Antibacterial.

Introduction

Microorganisms such as bacteria, filamentous fungi, and house dust mites may cause multiple infectious diseases. Although these microorganisms do not pose a threat to healthy people, persons with a compromised immunity are easily infected. Since the spread of the responsible microorganisms occurs mostly via airborne particles and water [1], it is important to treat water and air with efficient filters to improve the quality of life. Previously, antimicrobial filters using silver nanoparticles were reported. Unfortunately, silver nanoparticles have also been reported to be cytotoxic, whereas in the ideal case the filtration materials themselves should be nontoxic, and preferably biocompatible, especially when employed in the purification of domestically used air or water.

Hydroxyapatite (HAp) is a very promising candidate for application in filtration materials because of its excellent biocompatibility and nontoxic hypostasis with organic tissues [2], as well as its capacity to absorb bacteria and replication-competent human viruses [3]. Since the dimensions of microbes are small (ca. 0.5-5 μm in diameter for bacteria and 10-500 nm for viruses), it is very difficult to eliminate microbes using common air or water filters. So far, current techniques for processing porous HAp, such as starch consolidation and gel casting of foams, fail to yield pores with size smaller than macro pores [4, 5]. In the present work, air filters were coated with HAp by a spraying method. The surface of the coated filter was evaluated by SEM, and its E. coli and virus removal efficacy was studied.

*Corresponding author:
Tel : +82-55-350-5276
Fax: +82-55-350-5839
E-mail: scryu@pusan.ac.kr

Experimental

Preparation of HAp

HAp was prepared by reaction of $CaCO_3$ and H_3PO_4 using liquid state methods. In this study, the Ca : P ratio of the powder was determined to be 1.67. First, $CaCO_3$ was mixed with distilled water (120 g), and stirred for 5 hrs. H_3PO_4 (54 g) was slowly dropped into the $CaCO_3$ solution and stirring was continued for 12 hrs. After reaction the solution was dried at 100 °C, and the obtained dry powder was sintered at 1350 °C. The sintered powder was measured using an X-ray diffractometer (XRD) to observe the phase. The size of the HAp powder was reduced to nanometer dimensions by an attrition mill, which was loaded with 2000 g of balls, 300 mL of distilled water, and 300 g of sintered HAp powder. The total milling time was 12 hrs at a shaft speed of 400 rpm. HAp solutions were then made using the attrition mill and the HAp particles were deposited on the commercial filter in a compact coating.

Preparation of coating filter

Polyvinyl alcohol (PVA) was added to absorb the HAp particles on the surface of the untreated filter and the Hap/PVA solutions were spread on the surface of filter by spraying. It should be noted that the dipping or spreading method is amenable for mass production and reduces costs. After spreading, the filter was dried

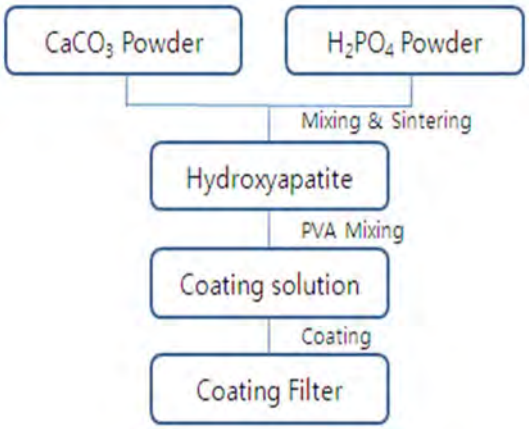

Fig. 1. Flow chart showing the process for producing the hydroxyapatite-coated filter.

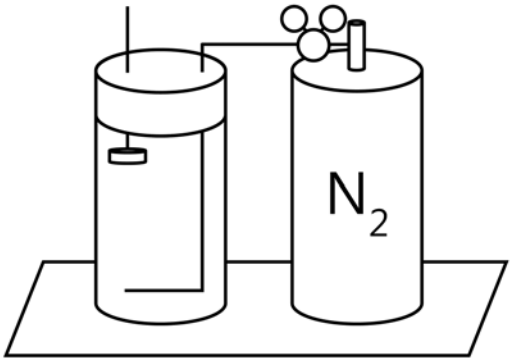

Fig. 2. Experimental set-up for testing the removal of E. coli in the air state by the HAp-coated filters.

at 100 ºC. The flow- chart of the process for producing the HAp-coated filter is shown in Fig. 1.

Characterization of the coated filter

The microstructure of the coated filter was measured by SEM. The samples were platinum-coated and observed at an accelerating voltage of 18 to 20 kV. The tensile strength and apparent density were measured according to the requirements of the Korean Standards Association. Since apparent density is closely related to the coated filter's pore size, we could estimate a change in pore size by the change in apparent density.

Removal efficiency of the coated filter

The removal efficiency of two kinds of infectious organisms, E. coli (Escherichia coli, KCCM 40880, gram negative) and the influenza virus (Influenza A virus, H_3N_2), were tested. In case of E. coli, the bacterium was incubated in tryptic soy broth for 24-48 hrs at 30 ºC. The liquid medium (100 mL) containing the E. coli was placed into an acrylic container that carried the Hap-coated filter on each end. Nitrogen gas was then injected into the container until all liquid medium had evaporated, after which the presence of E. coli on the surface of the HAp-coated filter was confirmed by SEM imaging. A schematic representation of the E. coli filtration process is shown in Fig. 2. For the virus testing, we investigated the removal rate of the filter by the plaque assay.

Antibacterial test

The antibacterial activity of the HAp disc was studied by the disc diffusion test. Following standard methods, agar was cast into Petri plates, and the plates containing the nutrient medium were evenly inoculated with E. coli (Escherichia coli, KCCM 40880, gram negative) that was incubated in tryptic soy broth for 24 hrs at 30 ºC. Disc samples of 10 mm in diameter were produced by pressing, under application of a pressure of 250 MPa, which was maintained for 20 sec. The pressed specimen was sintered at 1350 ºC for 4 hrs, at a heating rate of 5 ºC min^{-1} and a cooling rate of 4 ºC min^{-1}. The HAp disc specimen was then placed onto the agar plates and incubated at 30 ºC for 24-48 hrs, after which the area around the disc was examined for inhibition of bacterial growth. Additional antibacterial activity tests were conducted according to the requirements of the Korean Standards Association (KS K 0693 : 2011).

Result and Discussion

HAp coating of the filter

A spraying method was used for coating of the filter with HAp, which in principle allows mass production. The XRD pattern shown in Fig. 3 reveals that the HAp particles deposited on the comercial filter are crystalline. The peak positions and corresponding peak intensities in the XRD spectrum were consistent with the reference values of HAp as provided by the Joint Committee on Powder Diffraction Standards (JCPDS) (01-074-0566). The adhesion state of the HAp particles deposited on the filter was evaluated by SEM, indicating that the size of the grinded HAp powder was sufficiently small to be deposited on the filter fabric (Fig. 4). The HAp particles on the surface of the filter become more closely packed as spraying time increases. A cross-sectional SEM image of HAp particles in the filter is pictured in Fig. 5. It is clear that the HAp particles are uniformly deposited inside the filter and form a coating of at least 8 μm thick on the surface of the filter.

Apparent density

The apparent density and tensile strength of the coated filter is shown in Fig. 6. The apparent density of the coated filter was 369.25 g cm^{-3} and increased in value after coating with HAp solution. An increasing apparent density suggests that the weight of the filter increases, while the volume of the filter remains unchanged. The total volume of the pores is therefore reduced by coating with HAp solution. This consequently implies that pore size is reduced [6], which in turn

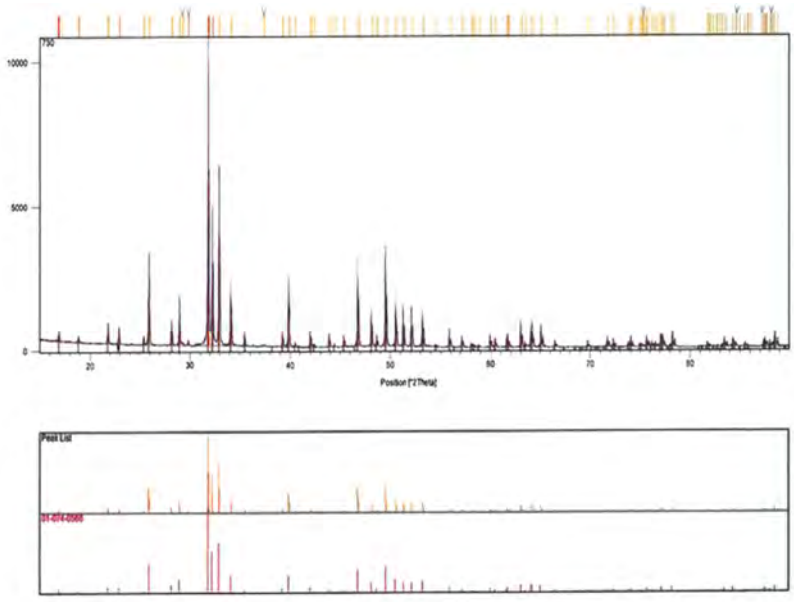

Fig. 3. XRD patterns of the HAp powder. (a) as-synthesised HAp and (b) the standard HAp spectrum provided by JCPDS.

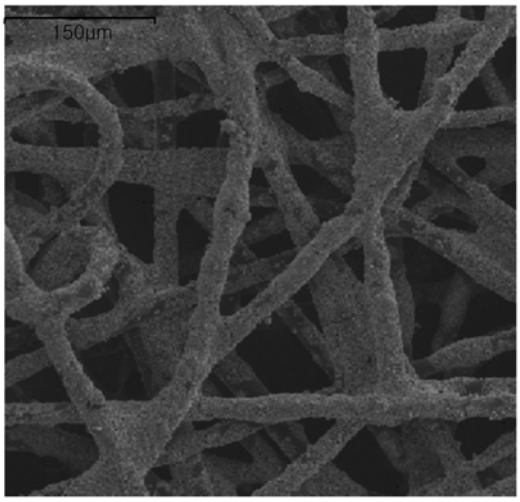

Fig. 4. SEM image of the HAp-coated filter produced by spraying. The filter was efficiently coated by the HAp solution.

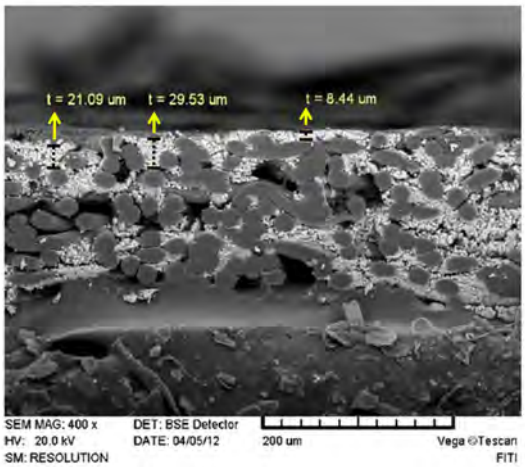

Fig. 5. Cross-sectional SEM image of the HAp-coated filter prepared by spraying.

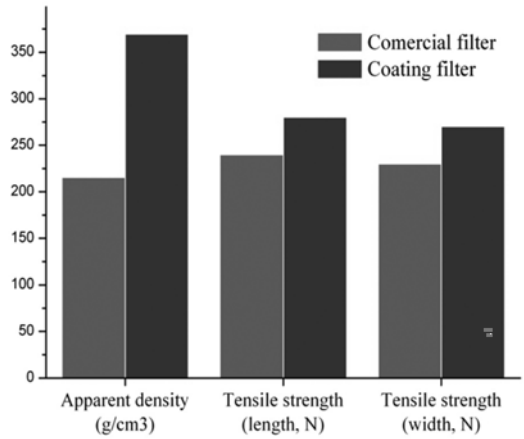

Fig. 6. Selected physical properties of the hydroxyapatite-coated filter.

influences the absorbability of the filter. The tensile strength of length and width was 280 N and 270 N, respectively, which is higher than that of comercial filters. No effect of the amount of coating on the tensile strength was observed.

Absorbability

The results of the E. coli filtering test are shown in Fig. 7 and Fig. 8, showing that the microstructure of the filter, as measured by SEM, is changed upon incubation with E. coli. The ellipsoidal features shown in Fig. 8 are reminiscent of the morphology of E. coli. Additional experiments were conducted to reveal that the ellipsoidal structure was indeed E. coli, as exemplified by the image in Fig. 9, which shows the agar plate after 24 hrs incubation. When comparing Fig. 8 and Fig. 9, a similar ellipsoidal structure can be observed indicating that the HAp-coated filter

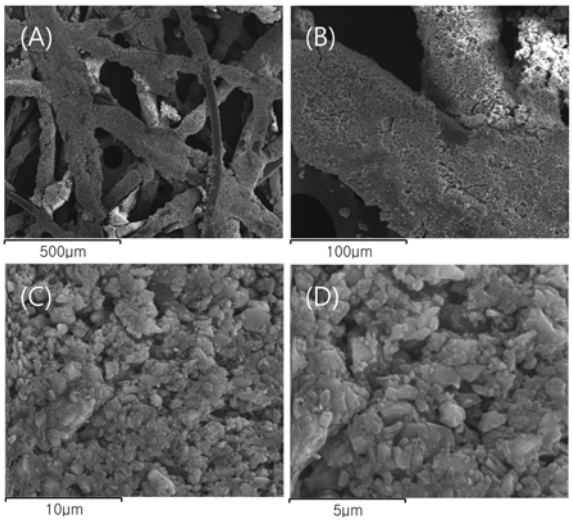

Fig. 7. SEM images showing the HAp-coated filter before filtration of E. coli.

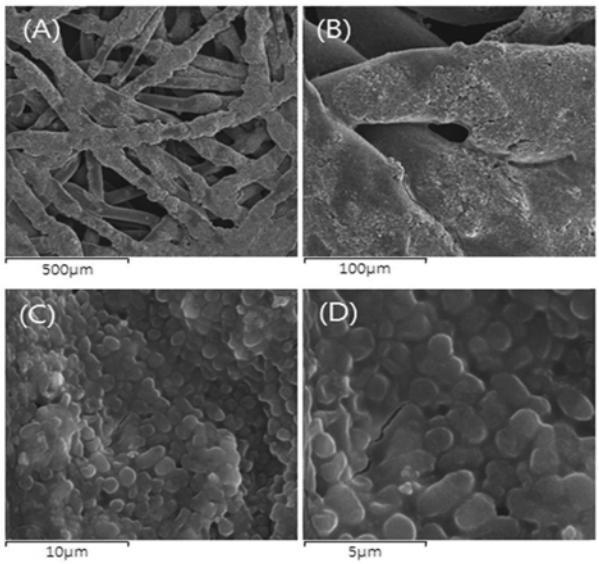

Fig. 8. SEM images showing the HAp-coated filter after filtration of E. coli.

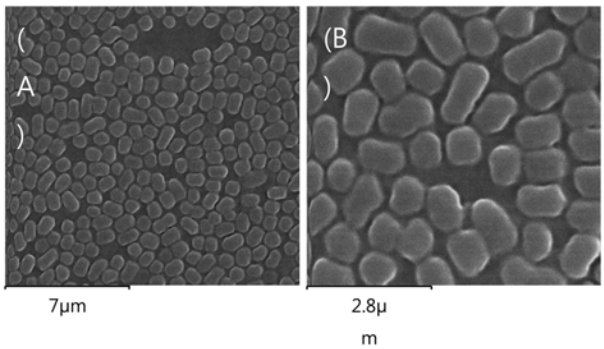

Fig. 9. SEM images showing E. coli on the solid agar medium.

effectively absorbs bacteria. The results of the plaque assay are displayed in Fig. 10, showing that a 1% solution of HAp particles displays between 90-99% absorbability.

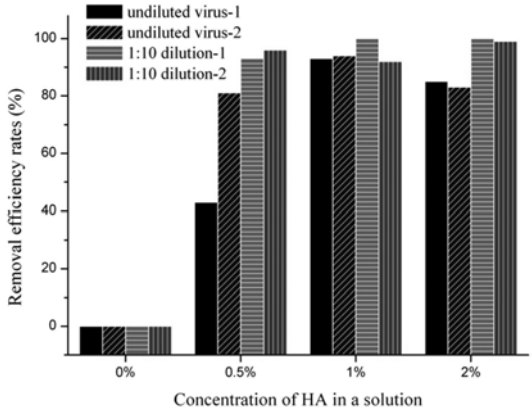

Fig. 10. Removal efficiency rates at various concentrations of HAp.

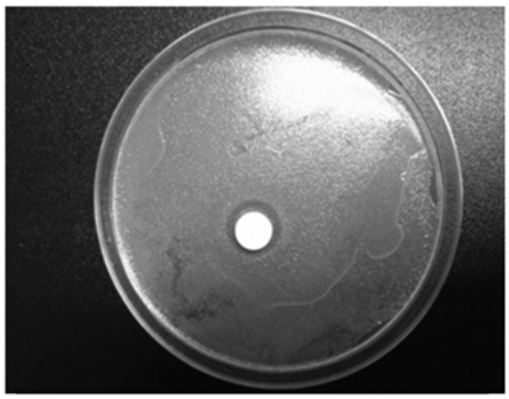

Fig. 11. Antibacterial activity of a pressed hydroxyapatite disc, measured via the paper disc method.

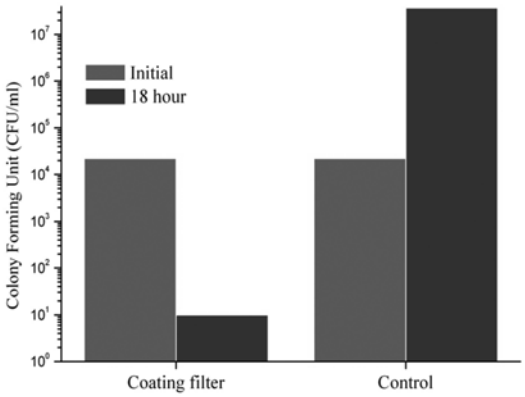

Fig. 12. Antibacterial activity test of the hydroxyapatite-coated filter, following the recommendations of the Korean Industrial Standards 0693 : 2011.

Antibacterial properties

The results of the antibacterial activity tests are shown in Fig. 11. The clear area around the HAp disc indicates the antibacterial effect [7]. The diameter of the clear zone is ca. 2.5 mm. The test results, as evaluated by the requirements of the Korean Standards Association (KS K 0693 : 2011), are shown in Fig. 12. In contrast to the control sample, which shows an increase in the number of colonies, the HAp-coated filter, showed a reduced number of E. coli colonies.

Conclusion

In this study, we prepared HAp-coated filters by a spraying method and investigated its physical and antibacterial properties. The HAp particles on the surface of the filter were more closely packed as the spraying time increased, while the tensile strength, as compared to the untreated filter, was enhanced. The reduced pore size of the filter, resulting from HAp coating, could enhance the filtration of E. coli. The absorbability of the filter was sufficient to completely remove E. coli as well as the influenza A virus in the liquid state. The antibacterial properties were also investigated by the paper disc method and in accordance with tests prescribed by the Korean Standards Association. The HAp-coated filter displayed 90-99% antibacterial removal efficiency.

Further studies should be performed to compare the filter's antibacterial properties in the liquid state to that in air. Finally, it will be important to investigate the structural basis of the antibacterial effect of the HAp-coated filter in the air state.

Acknowledgments

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