

Antiviral activity of multifunctional composite based on TiO₂-modified hydroxyapatite

Naruporn Monmaturapoj^{a,*}, Autcharaporn Sri-on^a, Wattana Klinsukhon^a, Kobporn Boonnak^b, Chureerat Prahsarn^a

^a National Metal and Materials Technology Center, 114 Thailand Science Park, Pathumthani 12120, Thailand

^b Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, 10400 Bangkok, Thailand

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ABSTRACT

An antiviral activity of TiO₂-modified hydroxyapatite composite (HA/TiO₂) had been investigated. The HA/TiO₂ composite (HA50:Ti50) was prepared by a solid state reaction method followed by calcination at 650 °C for 2 h. Phase formations and morphologies of the obtained HA/TiO₂ composite powders were determined using XRD and SEM. XRD result confirmed that HA/TiO₂ composite was successfully prepared. SEM revealed small crystals of anatase TiO₂ embedded in larger HA crystals. A strong antiviral activity against H1N1 Influenza A Virus was observed at 0.5 mg/ml concentration of the composite under the UV irradiation for 60 min. It showed the highest rate of reducing virus titer approximately more than 2 log/h. Results obtained from this study indicated that HA/TiO₂ composite could be a promising material to be used as antimicrobial filtration applications such as in face masks.

1. Introduction

Hydroxyapatite (HA, Ca₁₀(PO₄)₆(OH)₂) is a type of calcium phosphate ceramic. It is the most widely used in biomedical applications especially as an implant material [1–6]; as a filler in bone defects for orthopedic and maxillofacial surgeries and as a coating for metallic hip and knee prosthesis [7–19]. This is because it has composition similar to that of human bone and teeth. It is biocompatible, bioactive, osteoconductive, non-toxic to organic tissues, and able to adsorb bacteria and virus [13–17]. HA is capable of adsorbing molecules on its surface but not decomposing molecules, therefore, saturation will be reached over time [20, 21]. Meanwhile, titanium dioxide (TiO₂) is a polymorphic material (anatase, brookite and rutile) that has been extensively studied for solving environmental problems such as water and air pollutions because of its photocatalytic activity since first presented by Fujishima and Honda [21, 22]. Nano-sized titanium dioxide is one of the most popular photocatalysts employed due to its excellent photo-oxidation of organic compounds, strong oxidizing power under UV, chemical stability, non-toxicity and long-term stability against photo and chemical corrosion [22–27]. Although it can effectively decompose only substances that have come into direct contact, and the decomposition requires presence of light, especially in ultraviolet range [24, 26–28], TiO₂ had been widely employed for deodorizing and antibacterial under weak UV-light in living and working environments [28,

29]. Presently, TiO₂ has been known for its ability to destroy both Gram-positive and Gram-negative bacteria [30] including its efficiency against various viral species and parasites [31–33]. The mechanism of TiO₂ photochemical reaction with various reactive oxygen species (ROs) have been proposed and well-accepted [34–36].

Combining HA with TiO₂ offers advantageous characteristics of a new composite, such as HA adsorbs molecules, which will later be decomposed by TiO₂. This is well known as a multifunctional composite. Wakamura [36] proved that Ti⁴⁺ which replaces calcium cations in hydroxyapatite crystals has a similar antibacterial capability to that of titanium oxide. Our previous study had shown that nonwoven filter coated with HA/TiO₂ composite exhibited good bactericidal effect against both Gram-negative *E. coli* and Gram-positive *S. aureus* under ceiling mount fluorescent lights [37]. We also reported that the honeycomb structures of TiO₂-modified hydroxyapatite composite (HA/TiO₂), fabricated by extrusion technique, exhibited decomposition of bacteria approximately 50% after 2 h of UV exposure [38]. Matsunaga and coworkers firstly proposed the mechanism of photocatalytic decomposition of microbial through the direct photochemical oxidation of intracellular coenzyme A to its dimeric form [39]. This decreased cell respiratory activities resulting in cell death. Saito and coworkers [40] suggested that TiO₂ photochemical reaction caused disruption of cell membrane and cell wall of *Streptococcus*. This mechanism was also reported by Bak et al. [41]. The third mechanism is based on the ion

* Corresponding author.

E-mail address: narupork@mtc.or.th (N. Monmaturapoj).

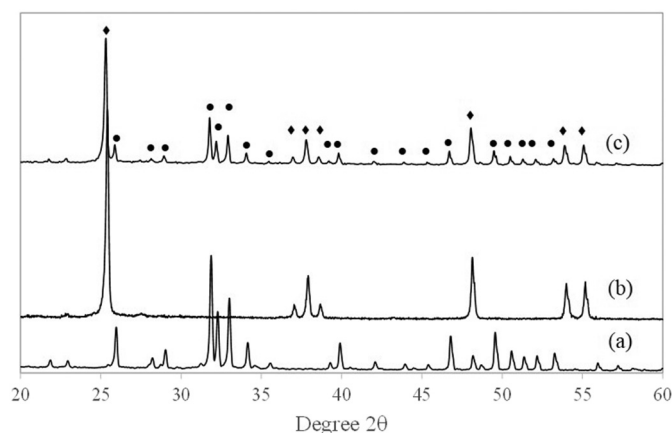


Fig. 1. XRD pattern of a) as synthesis HA, b) anatase TiO_2 , c) HA/ TiO_2 composite; ● Hydroxyapatite and ◆ anatase TiO_2 .

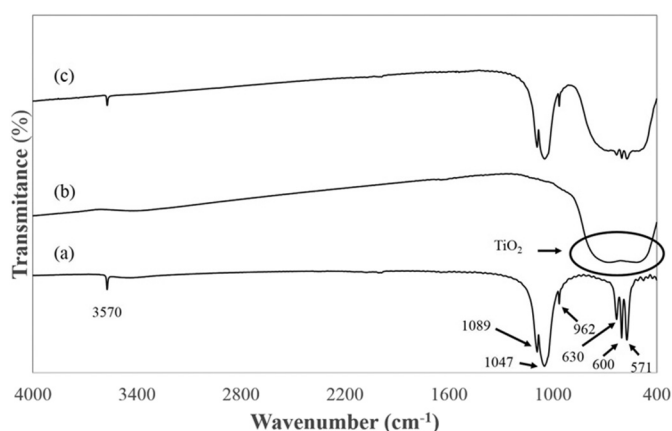


Fig. 2. FTIR spectra of a) as synthesis HA, b) anatase TiO_2 , c) HA/ TiO_2 composite.

induction of reactive oxygen species (ROSs) such that oxygen radicals are able to react with the components of the membrane and cell wall of bacteria. However, the mechanism of microbial photocatalytic decomposition has not yet been well-understood due to variation in microbial forms and their complexity in each case [28].

Most of previous studies had concentrated on antibacterial activity of TiO_2 and HA/ TiO_2 composite. Nowadays, not only bacteria can have effects on the respiratory system but also virus. Particularly, H1N1 Influenza A virus had spread worldwide and resulted in numerous deaths in the past few years. In the present work, HA/ TiO_2 composite was developed with an ultimate goal for antiviral activity. The HA/ TiO_2 composite was prepared and characterized, in comparison with HA and TiO_2 , separately. The antiviral activity of the composite powder against H1N1 Influenza A virus was investigated and discussed. The factors including composite dose, concentration of virus stock and UV-light activating time were varied to determine their effects on antiviral activity of the composite.

2. Experimental procedures

2.1. Preparation and characterization of hydroxyapatite-titania composite (HA/ TiO_2)

Pure hydroxyapatite (HA) and hydroxyapatite-titania (HA/ TiO_2) composite particles were prepared by a solid state reaction method as described in our previous work [37]. All raw materials were used as received. CaCO_3 (Carlo Erba Reagenti, Italy) and CaHPO_4 (Sigma-

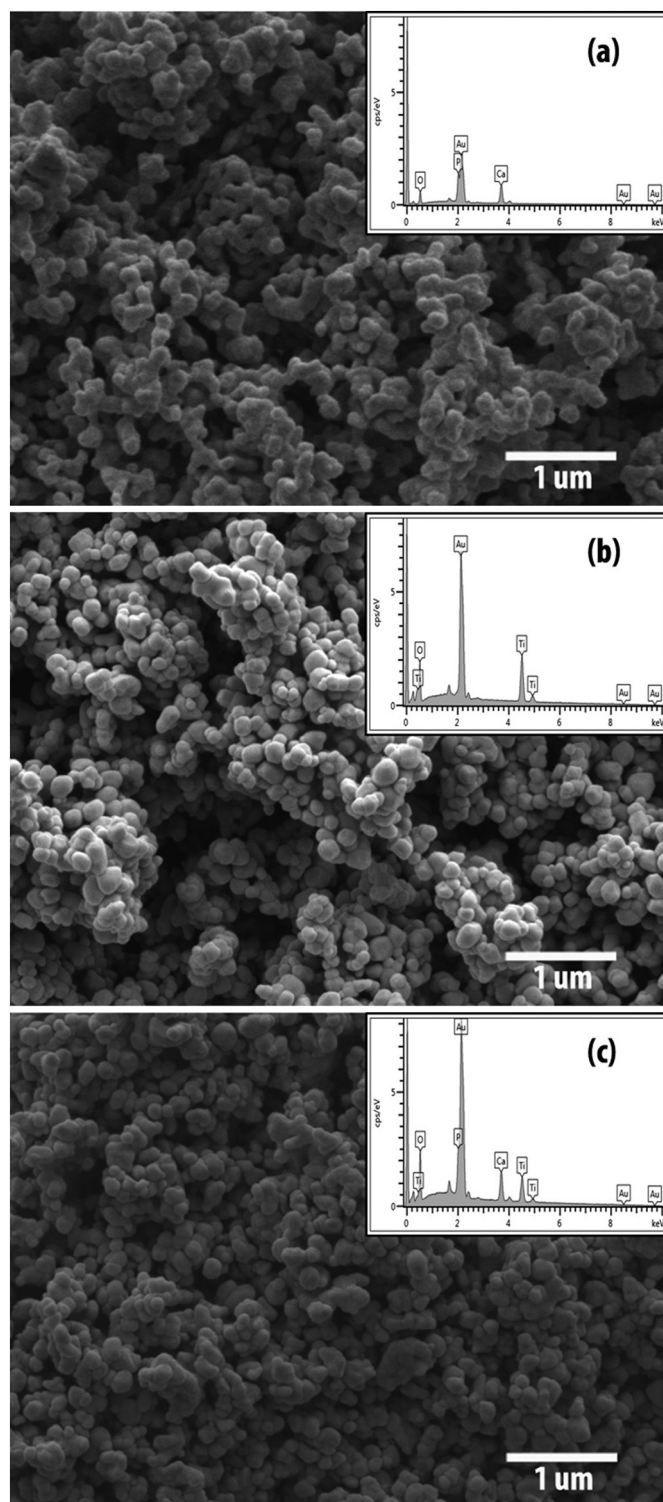


Fig. 3. SEM images and EDS traces of a) as synthesis HA, b) anatase TiO_2 , c) HA/ TiO_2 composite powders.

Aldrich, Germany) were ball-milled with zirconia balls in presence of deionized water as a media, followed by drying at 100°C . The mixture was then calcined at 1200°C for 2 h to produce pure hydroxyapatite powder. The obtained powders were ground and sieved through 106 μm mesh.

From our previous work [37], the HA/ TiO_2 composite of HA50: TiO_2 50 ratio exhibited the highest bacterial resuction. In this work, we thus selected the HA/ TiO_2 composite (HA50: TiO_2 50) for investigation on

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