The Preparation and Antimicrobial Effect of AgZrP/Nylon 6,10 Fibers Used as Dental Hygiene Materials

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Received 17 May 2010; accepted 30 March 2011
DOI 10.1002/app.34603

ABSTRACT: Silver zirconium phosphate (AgZrP) was incorporated into nylon 6,10 fibers by using a twin screw extruder to produce antimicrobial fibers. Monofilament fibers with various degrees of AgZrP loading were prepared by the melt-spinning process. The surface concentration of AgZrP particles was found to be dependent on AgZrP loading. A poor interface between AgZrP and the polymeric matrix was observed, however, it did not affect the drawn process. The presence of AgZrP particles did not disturb the structure and slightly effected to the mechanical properties of the nylon fiber. The fiber with the highest draw ratio showed the highest degree of polymer chain orientation, a higher tensile strength and a higher modulus. The antimicrobial effect started when the silver ion concentration was high enough, The AgZrP fiber showed the highest antimicrobial effect on *S. mutans*, *L. Casei*, *S. aureus*, and *C. albicans* at 10, 15, 10, and 15%, respectively. The AgZrP fibers showed the significant antimicrobial effect on three strains of microorganisms except *S. aureus*. Antimicrobial activities of AgZrP were demonstrated and the results showed that AgZrP incorporated into the matrix of nylon 6,10 fibers can improve their antimicrobial property. © 2011 Wiley Periodicals, Inc. J Appl Polym Sci 124: 4016–4024, 2012

Key words: fibers; inorganic materials; ion exchangers; polyamides; WAXS

INTRODUCTION

In dentistry, polymer becomes a popular material in a recent day. Polymer was applied to various applications, such as cosmetic treatment, restoration, and oral hygienic care. This is because of their outstanding characteristics, such as being light in weight, as strong as metals and easy to process. Except the useful properties of polymer, there are some required properties that are necessary for increasing the efficiency strength: biocompatibility, bioactivity, mechanical properties, hydrophilicity, roughness, and antimicrobial abilities.[1]

It seems antimicrobial ability is the most interesting in oral hygienic care because plaque, a sticky, colorless film of microorganisms colonizing on the dental surface, is the important key that leads to teeth cavities and gum diseases. For preventative, toothbrush was the most recommended piece of equipment for removing microorganisms, however, it is a large reservoir containing various kinds of microorganisms, as shown in many reports.[2,3] In particularly, these microorganisms can be transferred back into the oral cavity the next time it is used. It means that reducing of survival microorganisms on toothbrush tabs will reduce the microbial transference and reduce the risk of oral health diseases. In this research, antimicrobial agent was incorporated into nylon 6,10, which was aimed for using as antimicrobial toothbrush bristles. Nylon 6,10 were selected to use as nylon matrix because of it is softer, low water absorption material, better resistance to low temperature and has good dimensional stability when compared with another kind of nylon. Various kinds of materials have been used as disinfectants additive for polymer, such as quaternary ammonium, chitosan, titanium dioxide, and heavy metal. Among these material, silver has been widely used in various forms, such as metallic silver and silver nanoparticles since the silver ion (Ag^+) is considered to be effective against a broad range of microorganisms, including bacteria, fungi, and viruses, whereas it has a low toxicity to mammalian cells[4–9] and silver does not need exciting source for

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Contract grant sponsors: Office of the Higher Education Commission, Thailand, Thailand Research Fund (TRF), National Nanotechnology Center (NANOTEC).

Journal of Applied Polymer Science, Vol. 124, 4016–4024 (2012)

antimicrobial action like titanium dioxide.[10] The mode of action of Ag^{+9} was studied by Percival et al. and the action was simply summarized as follows: adsorption onto the bacterial cell surface; diffusion though the cell wall; binding to the cytoplasmic membrane; disruption of the cytoplasmic membrane; release of K^+ ions and other cytoplasmic constituents; precipitation of cell contents and the death of the cell.[9,11] Apart from pure silver which giving free silver ions, silver compounds, such as silver nitrate and silver sulfadiazine are useful in wound/burn therapy; however, silver compound with a controlled release, such as silver zirconium phosphate, have gained interest since a prolonged release can provide a longer antimicrobial effect. As a further matter, silver zirconium phosphate does not disturb the composite color as same as the silver nanoparticle, which turn the original color to yellow. The constant release of Ag^+ was found to have a higher clinical efficacy because it can provide a persistent presence of free silver ions in the local microbial environment.[4,12] Because of their powerful ability to inhibit microbes, silver releasing agents were incorporated into polymeric materials. Various coating methods, such as immersion, immobilization, and plasma modification, were selected for the modification process, whereas composite techniques have only appeared in a small number of reports. In this study, a composite technique was selected since it is easy to process. Silver hydrogen phosphate (AgZrP), which has high antimicrobial efficiency,[7] was incorporated into nylon 6,10 fibers to modify the antimicrobial efficiency.

MATERIALS AND METHODS

Preparation and characterization of AgZrP/nylon 6,10 fibers

All of the materials were used without any further purification. Silver zirconium phosphate (AgZrP, NDN, Thailand) containing 3000 ppm of silver and nylon 6,10 (cat. No. 5682005, Toray Industries, Japan) were mixed using a corotating twin- screw extruder (En Mach Co., Thailand, L/D = 40 : 1). The temperature was set at 100°C in zone 1, at 220°C in zones 2 to 7, at 245°C in zone 8, and at 250°C in zone 9 of the barrel of the extruder with a screw speed of 17 rpm. The composites extruded by the extruder were cooled down in a water bath and then cut into columned granules by a cutter. Subsequently, the composite granules were dried (vacuum, 70°C for 36 h) and extruded as fibers by a single screw extruder (Intro Enterprise Co., Thailand, L/D = 25 : 1). The spun fibers were drawn in 100°C glycerol at a drawing speed of 6–15 and a drawing ratio of 2-5. Fiber samples were characterized using a scanning electron microscope (*S*-2500 Hitachi Scanning Electron Microscopy, acceleration voltage of 15 kV), a tensile testing machine [INSTRON 5569 at the Research and Development Centre for Thai Rubber Industry (RDCTRI), Thailand] and a 2D X-ray diffractometer (Rigaku Image Plate equipped with RUH3R rotating anode, X-ray generator and *R*-axis IV^{2+} 300 mm × 300 mm image plate detector, Cu Ka L = 1.54Å, beam of 0.3 mm was produced at a voltage of 50 kV and a current of 100 mA).

Determination of antimicrobial activity

Culture preparation

Streptococcus mutans $KPSK_2$, *Lactobacillus Casei* ATCC 6363, *Candida albicans* ATCC 10231, and *Staphylococcus aureus* ATCC 6538 were used to determine the antimicrobial properties of the AgZrP/nylon 6,10 fibers. All bacteria and yeast were grown on brain heart infusion (BHI) agar and sabouraud dextrose agar obtained from BBL™, respectively. Each of them was suspended in phosphate buffer saline (PBS) and adjusted the turbidity equivalent of McFarland 0.5.

Antimicrobial activity

Five ml of saliva was mixed with 5 mL of each microbial suspension to improve microbial adherence. A volume of 150 μL of inoculum was transferred to 96-well plate. Then, 5 bundles of 20 fibers of 0, 5, 10, and 15% AgZrP/nylon 6,10 were immersed in the microbial suspensions at 37°C for 1 h. The unattached cells were washed with PBS 3 times and the adhered microoganisms were eluted from the fiber surface by shaking vigorously for 1 min in PBS using a vortex mixer. The eluted microbial suspensions were diluted 10-folds and cultured on BHI or sabouraud dextrose agar. The numbers of surviving microorganisms were counted as colony forming units per surface area of fiber (CFU/cm^2). To simulate daily use of a toothbrush, the AgZrP/nylon 6,10 fibers were immersed in microbial suspension for 5 min twice a day and repeated for 5 days to simulate daily use of a toothbrush. The fibers were kept in a sterilized condition during the experiment. The numbers of bacteria survivors were determined at 5 h after the first and last inoculation by the same process as aforementioned. Neat nylon 6,10 fibers were used as a control throughout the entire experiment. The data were analyzed by one-way ANOVA with LSD post hoc by SASW statistics 18.

RESULTS AND DISCUSSION

The AgZrP was dried before being mixed with the nylon 6,10 fibers to prevent aggregation. It had a

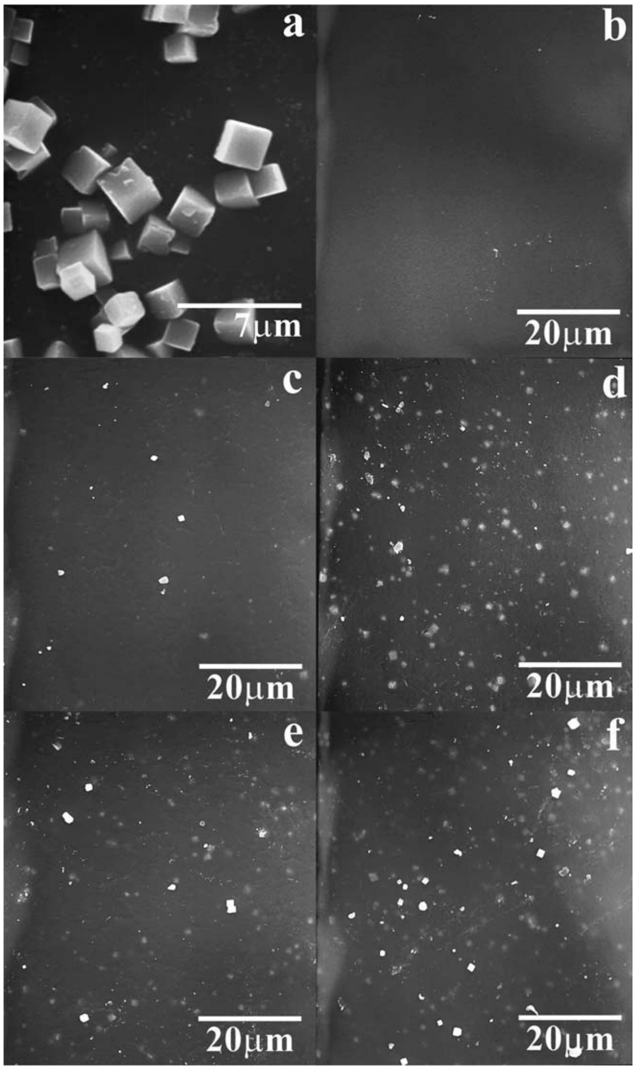

Figure 1 SEM images of silver hydrogen zirconium phosphate (AgZrP) (a), 0% (b), 1% (c), 5% (d), 10% (e), and 20% AgZrP composite pellets (f).

cubic shape with an average size of 2 μm, as shown in Figure 1(a). The AgZrP showed a very good dispersion in the polymeric matrix, as can be seen from the SEM images in Figure 1(b–f). The ratio, in weight percent, of the AgZrP in the composites were 1, 5, 10, and 20% and the images of these pellets are shown in Figure 1(c–f), respectively. Most of AgZrP particles were embedded in the polymer matrix due to the large difference in the ratio of polymer to AgZrP. The volume distribution of the AgZrP particles depended on the amount of AgZrP loading and it was found that the AgZrP showed a fairly good coverage on the skin of the polymer pellet. It is desirable to have AgZrP on the skin of the polymer to promote the release of silver ions. Otherwise, the release of the embedded AgZrP may be hindered and the antimicrobial activity might be delayed due to the silver ions having to travel to the polymer surface. Figure 2 confirms the existence of AgZrP in nylon6,10 matrix. Both of (a) and (b) are captured from the same area. 5 kV (a) and 15 kV (b) images showed the information of top and under surface, respectively. There are a lot of AgZrP particles in Figure 2(b); however, there are some of them presented in Figure 2(a). It was concluded that almost AgZrP particles embedded in the thin layer of nylon.

The AgZrP/Nylon 6,10 pallets were fed into the single screw extruder to prepare the fiber. The fiber was first spun into the monofilament fiber. The draw ration of the fiber was controlled by the speed of the leader to the follower. The temperature of the bath was kept at 100°C. The maximum draw ratio, which was achieved was 5 for all pure nylon 6,10 fibers and up to 10% AgZrP fibers. The maximum drawn ration decreased to 4 for the 15% AgZrP composites. Formation of the fibers was not hindered by the presence of AgZrP at a percentage loading less than 10% and this sample was chosen for further investigation. Another interesting feature was the formation of a microvoid which appeared on the AgZrP fiber surface after drawn processing, as shown in Figure 3. This microvoid formed because of a poor interaction between the AgZrP particles

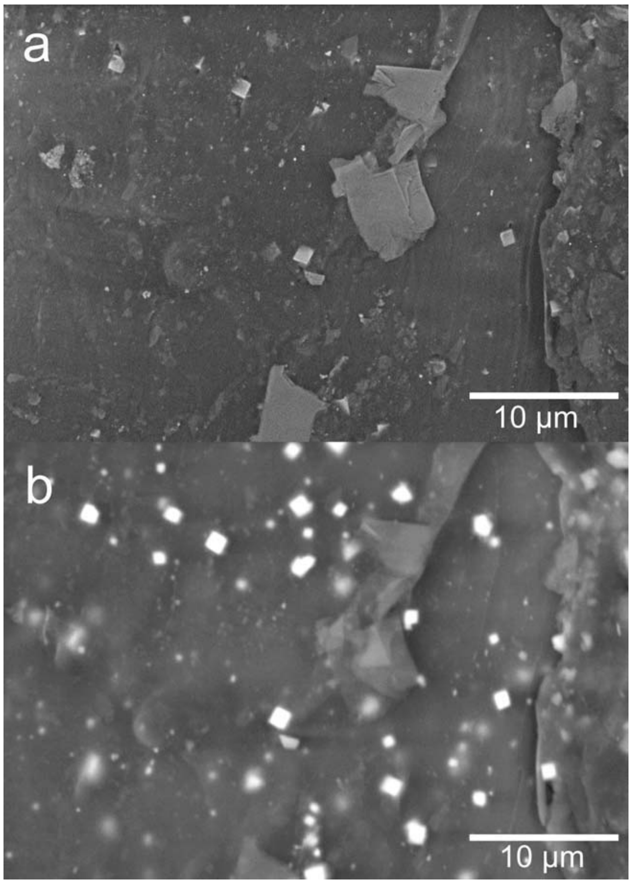

Figure 2 SEM images AgZrP/nylon 6,10 fibers [DR = 0] (a) was captured by low vacuum SEM at 5 kV and (b) was captured at 15 kV.

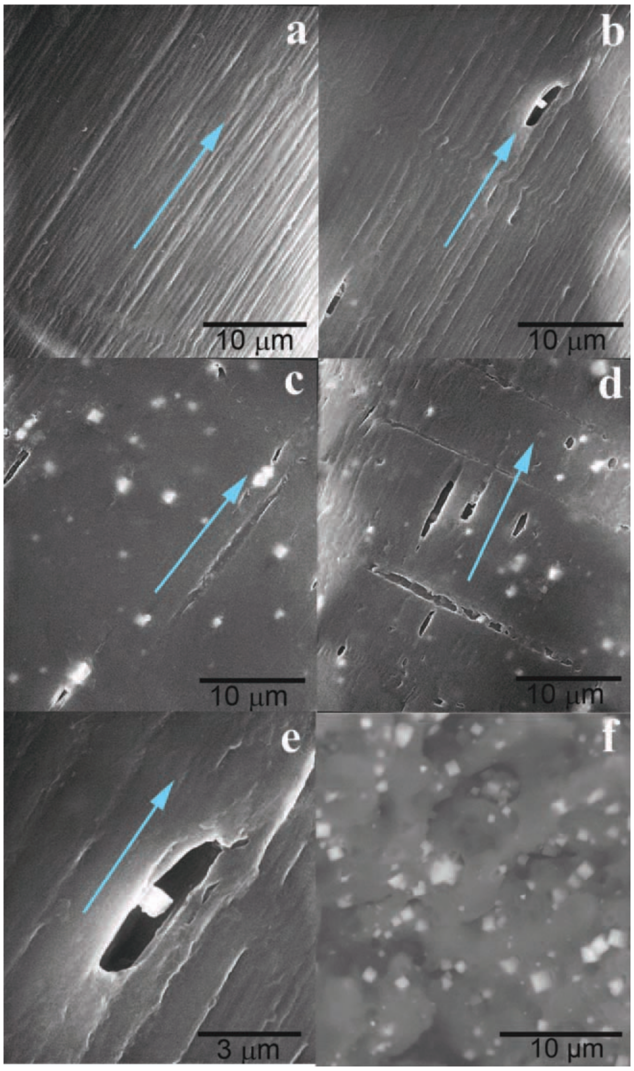

Figure 3 SEM images AgZrP/nylon 6,10 fibers [DR = 4] (a) 0%, (b) 1%, (c) 5%, and (d) 10%; (e) high magnification image of microvoid and (f) x-section of 10%. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and the nylon matrix. The formation of the microvoid can be reduced by changed the drawing condition to a higher temperature. However, the formation of this void was intentional to promote the release of silver from the AgZrP particle. The polymer matrix acted as the glue, it did not bind to the AgZrP particles, hence, the AgZrP particles were only trapped in the melted polymer matrix. There were some AgZrP particle present on the surface, however, there were a lot of unexposed AgZrP particles inside the fiber, which can be observed from the cross sectional image in Figure 3 (e,f). A more effective approach in producing the fiber would be to use a multicomponent extruder, where only the skin layer contained AgZrP to save on the costs of material preparation,[13] as this should reduce the amount of the AgZrP required.

The 2D wide-angle X-ray diffraction patterns illustrated in Figure 4 show that both the nylon fibers and the AgZrP/nylon 6,10 fibers exhibited lower angles of reflection at $2\theta \sim 13^\circ$, which corresponded to the amide–amide distance, and higher angle reflections at $2\theta = 28^\circ$. Figure 4 also show an identical pattern of nylon 6,10 to the one reported in the work by Fuller et al.[14] This result shows that two reflection planes occurred in the diffraction. The 002 arcs were the most intense and concentrated on the meridian while the 100 reflections were also strongly arced on the equator which were normal and parallel to the material planes, respectively.[13,15] The XRD patterns of the AgZrP/nylon 6,10 fibers show the combined diffraction patterns of AgZrP and nylon 6,10. The nylon peaks can clearly be seen while the peak from AgZrP does not show any preferred orientation. The presence of AgZrP particles in these nylon 6,10 melted extruded fibers did not alter the crystalline structure of the nylon. The only factor that altered the crystalline structures of the nylon was the drawn effect and this corresponded to the resulting mechanical properties. The higher drawn ratio showed the better crystal orientation of the nylon 6,10 matrix. The intensity of two patterns including the AgZrP particles and the nylon 6,10 matrix showed the ratio of AgZrP particles and the nylon matrix. The results showed that while the pattern of the AgZrP which embedded in the nylon matrix showed the higher intensity, the intensity of the nylon 6,10 patterns decreased, as shown in Figure 5. This confirmed the increase in the value of AgZrP particles.

Figure 6 shows the tensile mechanical properties of the neat nylon and the AgZrP/nylon fibers. Both the neat nylon and the AgZrP/nylon composite fibers showed the same trend in all mechanical properties. There were slight changes in the slopes of tensile strength and an elongation at the maximum load was observed, except for the 15% AgZrP/nylon fiber. The 15% AgZrP/nylon fibers showed different slopes of tensile strength and elongation properties, whereas the modulus of the 15% AgZrP/nylon fiber showed the same incline as for the other fibers. These results confirmed that the AgZrP particles slightly changed the mechanical properties of the fiber structures; however, it has less influence than the drawn effect. It was found that when the drawn ratio increased, the tensile strength and the modulus of the fibers increased, while in contrast, the elongation of the fibers decreased.

Antimicrobial activity

Four important microbial strains were chosen for antimicrobial test: *S. mutans*, *L. casei*, *S. aureus*, and

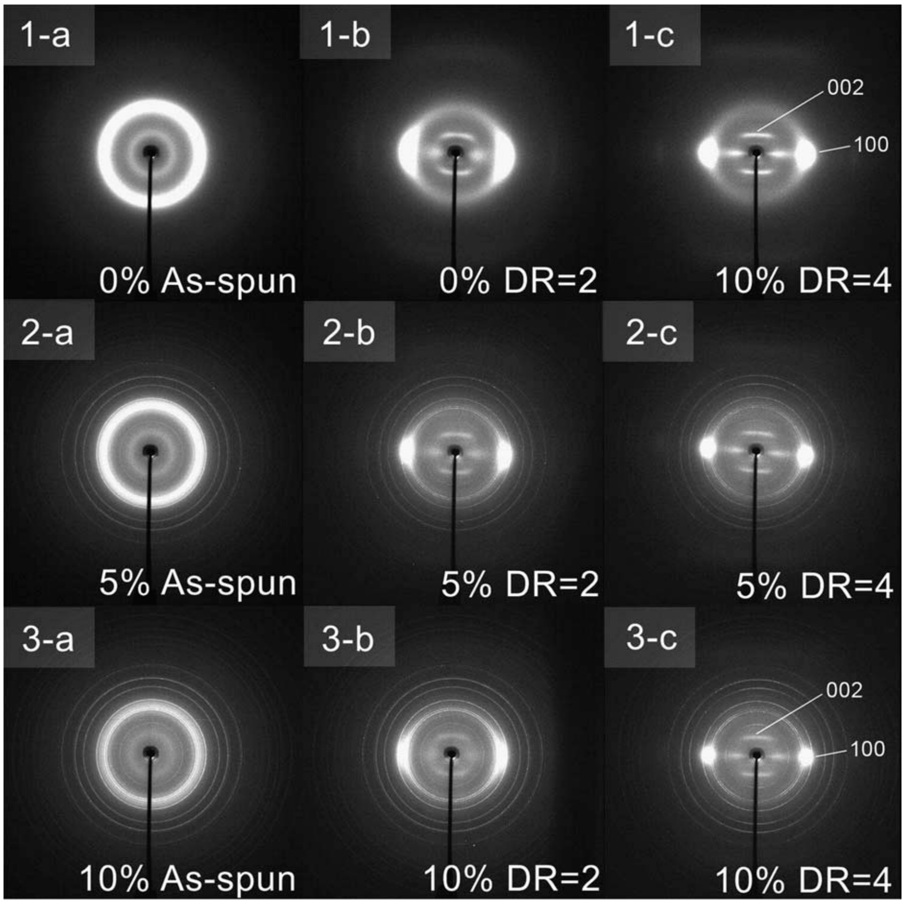

Figure 4 X-ray diffraction patterns of neat nylon fibers (1-a, 1-b, 1-c), 5% AgZrP/Nylon 6,10 fibers (2-a, 2-b, 2-c) and 10% AgZrP/Nylon 6,10 fibers (3-a, 3-b, 3-c) of which (a) is As-spun, (b) is DR = 2, and (c) is DR =4. Fiber direction is along the meridian.

C. albicans. Because they are the main microoganisms involved in tooth and gum diseases. Tooth decay is an infection disease commonly found in the oral cavity. It is caused by *S. mutans* and *Lactobacillus* spp.[16] *C. albicans* is an opportunistic fungi which is often found to be a causative agent of candidiasis in immunocompromized patients.[17] In addition, yeast cells can coaggregate and make synergistic relationship with pathogenic bacteria, such as *S. aureus* which lead to a mixed infection.[18]

Table I shows microbial survival on AgZrP/nylon 6,10 fibers as a function of AgZrP loading for each microbial strain. The surviving microbes were recovered from both neat and AgZrP/nylon 6,10 fibers. The results showed that the amount of *S. mutans* was decreased on fiber surfaces loaded with AgZrP and the maximum anitmicrobial ability appeared at $\geq$ 5% AgZrP (% reduction $\sim$ 70%).

Comparison with the other test microorganisms, it was found that *S. mutans* is sensitive to AgZrP concentration than *L. casei, S. aureus,* and *C. albicans,* which showed optimum antimicrobial concentration at 15, 10, and 15%, respectively. It seems high concentration like 15% AgZrP showed good performance in antimicrobial ability; however, the physical properties rather dropped. Hence, the optimum concentration for all test strains is in the range of 5–10%, and it seems that AgZrP/nylon 6,10 fibers showed higher antimicrobial effect on *S. mutans* and slightly afforted on the other strains. There were no clear reports about the killing mechanism by Ag ions, however, it is believed that the difference in effects on different strain of microorganisms are from the silver-cell wall binding potential. These were supported by Hamada et al., Knox et al., Strominger et al., and Chaffin et al.[19–22] They found that there are difference in ratio of the chemical components on the difference strain cell walls. Appending with Shoeib report which reporting about Ag ions energy binding of amino acid,[23] it is possible to conclude that the microbial-killing potentials of Ag ions depend on silver-microbial cell wall binding ability.

Although AgZrP/nylon 6,10 composite fiber did not show high antimicrobial ability, it is useful for using as dental hygiene material because it can reduce the microbial colonization on the fiber surface which leads to microbial transference and oral diseases to the patients, especially low immunity

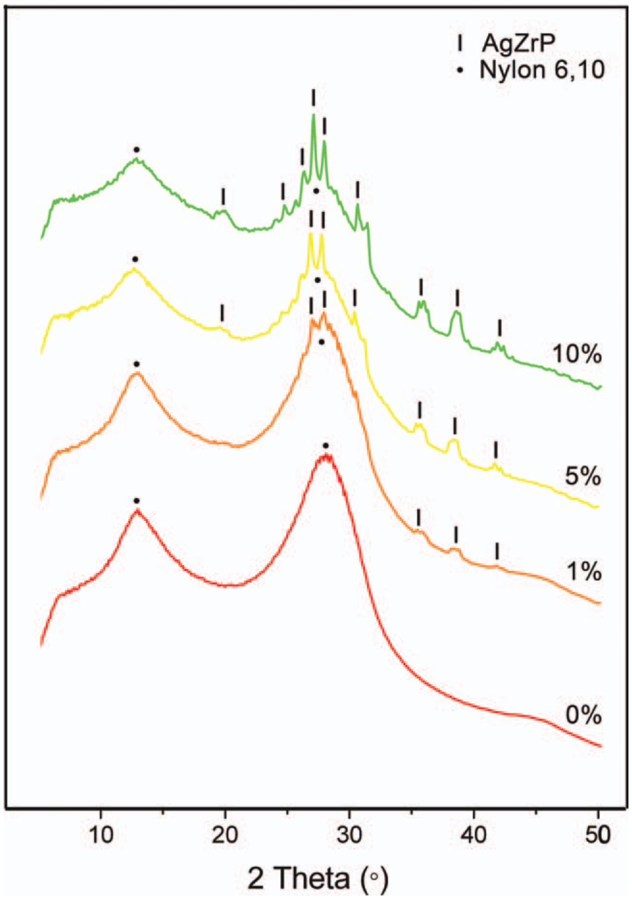

Figure 5 Photometer curves of integration of redial parts of X-ray diffraction patterns of AgZrP/nylon 6,10 fibers at drawn ratio = 4. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

and poor hygienic patients. Although the antimicrobial ability of fibers is strong only on *S. mutans*, AgZrP/nylon 6,10 fibers will still be an interested material because *S. mutans* is the most important key for tooth caries disease, which the most popular problem in dentistry. The carry process starts when *S. mutans* bind to the preferable molecules, which are the extracellular polysaccharides. Then *S. mutans* release certain acid to destroy a small spot on the dental surface and the damages enlarge by *Lactobacilli* spp.[19] Hence, inhibition of *S. mutans* is the most important key to reduce the initiator of dental caries problems.

Form Figure 7, the presence of the AgZrP was believed to promote adhesion of the bacteria to the fiber surface. At the 1% loading of AgZrP, all of the data, except for *S. mutans*, showed that the presence of AgZrP in nylon fibers increased the number of microbe survivors on the fiber surfaces. It might have been possible that AgZrP particles promoted microorganism adherence to fiber surfaces through a variation in the surface charge of microbes. This variation is dependent on microbe species and is influenced by the growth medium, age, and surface structure, the report showed that almost all bacteria in aqueous suspensions are always negatively charged.[24] Hence, Ag^+ ions which are released from AgZrP fibers not only bind to damaged cell walls of microbes but also the attractive force between the Ag^+ ions and the negative charges on microbe cell

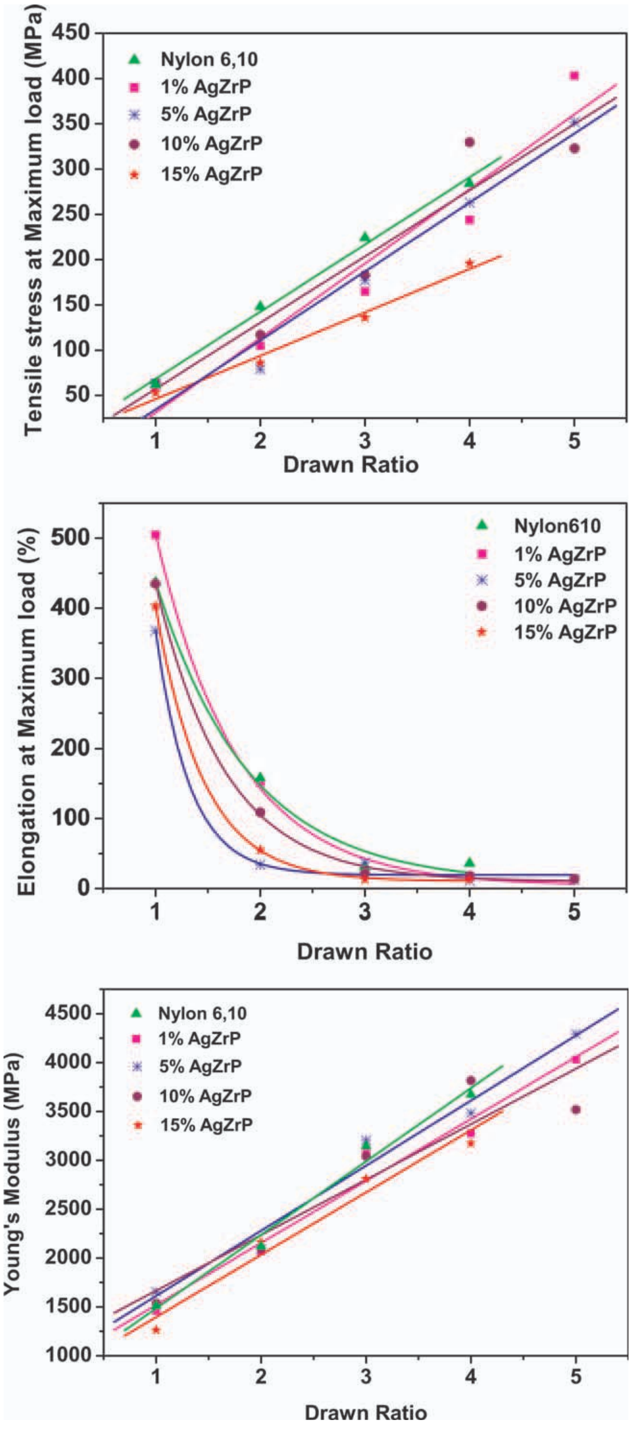

Figure 6 Tensile testing results of neat nylon and AgZrP/nylon 6,10 fibers: (a) tensile stress at maximum load, (b) elongation at maximum load, and (c) Young's modulus. [Color figure can be viewed in the online issue, which is available at www.onlinelibrary.wiley.com.]

TABLE I
Reduction of Microbial Survivors on AgZrP/Nylon 6,10 Fibers

	% Reduction of microorganisms on AgZrP/nylon 6,10 fibers			
Microorganisms	1%	5%	10%	15%
S. mutans	22.82	73.58[a]	74.06[a]	71.96[a]
L. casei	NR	44.56[a]	16.80	56.94[a]
S. aureus	NR	14.37	32.23	NR
C. albicans	NR	NR	NR	100.00[a]

NR = No reduction.

[a] Mean difference is significant with 0% AgZrP. (P-value ≤ 0.05).

walls promote adhesion of microbes to the fiber surfaces. It is possible that the Ag^+ rate of release for the 1% loading of AgZrP was lower than the MIC. Consequently, the microbe–microbe binding rate had a greater influence than the bacteria-killing rate for the 1% AgZrP loading (Fig. 8). Moreover, the SEM image in Figure 3 supported the idea that 1% loaded AgZrP fibers had a greater surface roughness, which increased the rate of bacterial colonization. This has been supported by many researchers. For example, Baker and coworkers reported that roughening the surface of either glass or polystyrene with a grind-stone greatly increased the rate of bacterial colonization in a river environment. These studies indicated that surface roughness influenced bacterial adhesion and this was also the conclusion reached by the scientists. This was due to a greater roughness being found on larger surface areas and depressions in the rough surfaces providing more favorable sites for colonization.[24,25]

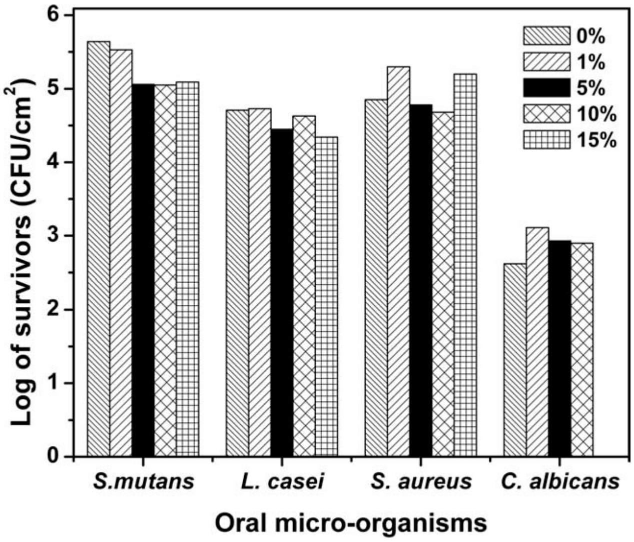

Figure 7 Antimicrobial ability of AgZrP/nylon 6,10 fibers on four types of microbes

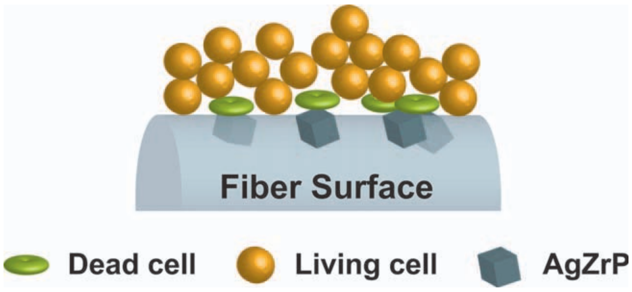

Figure 8 Microorganisms/silver ions adherence model. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

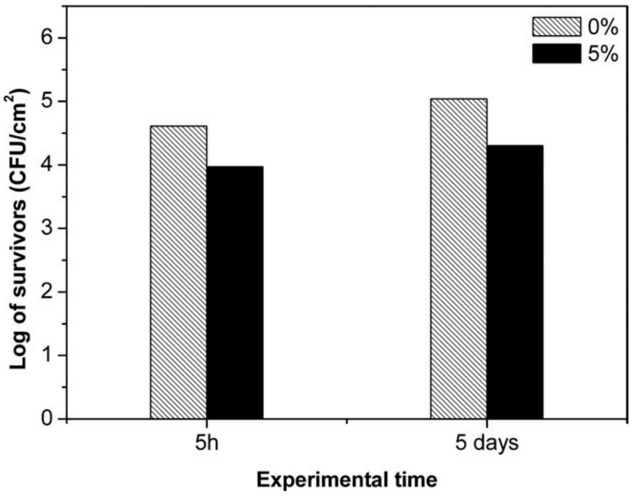

Figure 9 Antimicrobial activity of AgZrP/nylon 6,10 fibers on *S. aureus*, which was inoculated 5 min twice a day.

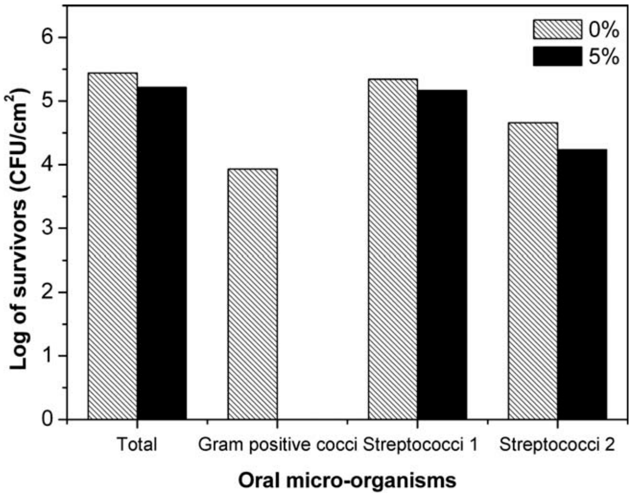

Figure 10 Antimicrobial activity of AgZrP/nylon 6,10 fibers on oral microbes at 5 h after 5 min of inoculation.

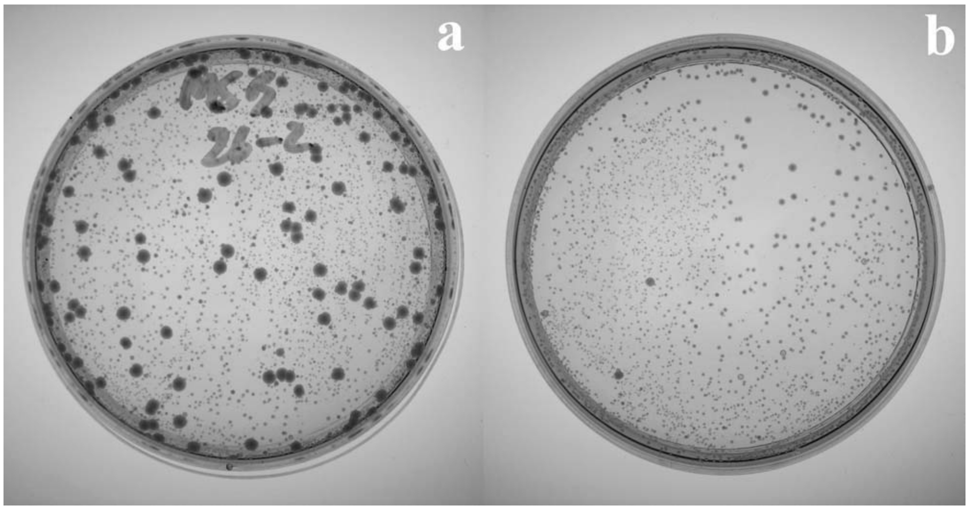

Figure 11 Antimicrobial activity of AgZrP/nylon 6,10 fibers on oral microbes: 0% AgZrP fibers (a) and 5% AgZrP fibers (b), at 5 h after 5 min inoculation.

It can be concluded that 5% loading of AgZrP was the optimum concentration because this showed antimicrobial effects on *S. mutans* and *L. Cesei*. However, it did not show a significant effect on *C. albicans*.

The AgZrP/nylon 6,10 fiber was further investigated by inoculating 5% AgZrP/nylon 6,10 fibers with *S. aureus* and human saliva to represent Gram positive bacteria and oral microbes, respectively. The antimicrobial activity of 5% AgZrP/nylon 6,10 fibers is shown in Figure 9. The result showed that the number of *S. aureus* on 5% AgZrP/nylon 6,10 fibers were reduced by ~ 80% over the period from 5 h to 5 days. This suggests that AgZrP/nylon 6,10 fibers had prolonged antimicrobial properties, which is good for long-term use of this material. As shown in Figure 10, the surviving microbes were classified into three groups that included gram positive cocci bacteria and two groups of Streptococci. It was found that 5% AgZrP/nylon 6,10 fibers reduced Streptococci from log 5 by ~ 40% and completely removed gram positive cocci bacteria from log 4. Figure 11 also shows the antimicrobial activity of 5% AgZrP/nylon 6,10 fibers on the mixed culture of oral microbes. This corresponded to the results obtained for *S. aureus* because it represents high antibacterial effective on gram positive cocci bacteria. Although the results of the mixed culture after 5 h are in accordance with the results of the pure culture of *S. aureus* after 5 h, the results after 5 days do not correspond. It was found that three groups of microbes that were found after 5 h disappeared and a new species of bacteria grew over the fiber surfaces. The possible reason is the nature of bacteria, as explained by An and Friedman in terms of an imbalance of controlling factors.[24] This report showed that in an environment where more than one species of bacteria is present, when an imbalance of the controlling factors occurs one bacterium may overgrow and crowd out other bacteria.

CONCLUSIONS

The AgZrP/nylon 6,10 fibers were successfully prepared by blending and the melt-spinning method. The presence of AgZrP particles increased the antimicrobial property of the fibers without interfering with the nylon structure or interrupting the processing of the fibers. The optimum concentration was 5–10% of AgZrP. The mechanical properties of the AgZrP/nylon fibers did not differ from the mechanical properties of neat nylon fibers. In regard to long-term use, it was found that the fibers still had antimicrobial properties after 5 days. However, the studied microorganisms in the mixed culture experiment were crowded out by a new species of bacteria.

The authors thank Associate Professor Dr. Jirundon Yuvaniyama and his students for their kind assistance in the XRD experiment, and also, Mr. Worapong Jamratpongvikai for his kind assistance in SEM experiment.

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