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Development of a transparent, non-cytotoxic, silver ion-exchanged glass with antimicrobial activity and low ion elution

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ABSTRACT

We investigated the antimicrobial, cytotoxicity, skin irritation, and ion elution behaviors of glass doped with silver ions with respect to its application to electronic equipment such as phones and tablet screens. The microbes tested were *Escherichia coli*, *Staphylococcus aureus*, and *Penicillium funiculosum*. $AgNO_3$ powder was spread on both sides of aluminosilicate glass, and it was heated to 250–280 °C for 10 min. Under optimized heating conditions (260 °C, 10 min), the antimicrobial activity of ion-exchanged glass against bacteria and fungi was over 99.9% after 24 weeks. The glass failed to irritate the skin of experimental animals and was considered non-cytotoxic. The maximum amount of Ag ions that were eluted from the ion-exchanged glass into drinking water was measured at $0.037 \pm 0.003\ \mu g\ L^{-1}$, an amount which is several orders of magnitude below the standard limit of $0.1\ mg\ L^{-1}$ in drinking water. Ag ion-exchanged glass had characteristics suitable for use as a display screen, such as a light transmittance of 90% and a surface roughness of 0.704 nm. Our findings suggest that glass doped with silver ions is more hygienic than non-doped glass is, and should be applied to display screens and glassware.

1. Introduction

In the last decade, the use of glassware, and of display screens in applications such as cellular phones and touch panels of medical devices, has risen quite significantly, and there have been many studies about the adverse effects of certain bacteria and fungi on the human body. The reproduction of such potentially harmful microbes depends on environmental factors, such as temperature, humidity, and contamination (i.e., transfer of microbes between surfaces) [1–3]. In particular recent studies have suggested that *Staphylococcus aureus* (gram-positive) and *Escherichia coli* (gram-negative), which can cause dermatitis, pneumonia, and septicemia, have been detected on the glass surfaces of mobile phones [4–6]. In addition, *Penicillium funiculosum*, the spores of which can cause asthma and rhinitis, has been found on display screens [5]. In order to remove harmful bacteria and fungi, washing of product surfaces using isopropyl alcohol [5,6] is encouraged, but this method does not confer long-lasting antimicrobial activity. Therefore, products with potent antimicrobial activity and durability have recently been produced by incorporating Ag and Cu ions into the glass surface as nanosized particles, thus providing a large specific surface area [7–10].

The mechanism for the antibacterial action of silver ions is already well known. The interaction between Ag ions and bacteria leads to the production of reactive oxygen species [11], which suppress microbial proliferation and viability by causing oxidative stress and cell damage [11,12]. Thus, with a small particle size and large specific surface area, the antimicrobial property of the material will be enhanced because of an increased contact area between the Ag ions and bacteria. In comparison, Cu nanoparticles have less antimicrobial activity, lower biocompatibility, and shorter antibacterial durability compared with Ag nanoparticles [4,10,13]; therefore, we used Ag ions in this study. Ag ions are often used to confer antimicrobial activity to materials, including nanoparticles, thin films, and bioactive glass [14–17]. However, in recent years there have been growing concerns about the use of Ag ions in materials, due to problems such as a reduced adhesive strength of thin films, skin irritation by eluted Ag ions, and mammalian cytotoxicity and genotoxicity [11,15,18]. Ag ions have toxic properties in the human body, and so the World Health Organization (WHO) has restricted the maximum safe concentration of Ag ions to $0.1\ mg\ L^{-1}$

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[19]. However, there have been only few studies relating to Ag ion elution from antibacterial glass products.

The conventional ion exchange method [20,21] is conducted by using the molten salt of $AgNO_3$ powder diluted in deionized water. The glass specimen is then heated in the presence of the diluted molten salt at 300–450 °C for 4–12 h, during which time the ion exchange occurs. However, instead of this general method, we used a new method called spreading ion exchange, in which the $AgNO_3$ powder was spread over both sides of the glass and then the glass was heated at 250–280 °C for 10 min; this method considerably increases the speed of the ion exchange process. In general, during ion exchange, the diffusion rates of Na and Ag ions vary according to the concentration of Ag ion and the glass composition. The aluminosilicate glass commonly used for display screens and glassware has better diffusivity of movable cations than other kinds of glass, because the presence of Al_2O_3 in aluminosilicate glass increases nonbridging Na^+ ions and decreases the activation energy for diffusion [20].

In this study, the Ag ions were exchanged with alkali ions at the aluminosilicate glass surface with a short processing time by using the spreading method. The antimicrobial properties of the ion-exchanged glass against *E. coli*, *S. aureus*, and *P. funiculosum* were evaluated. The durability of the antimicrobial effect and its relation to the penetration depth of the Ag ions were confirmed by taking measurements from each individual glass specimen between 1 and 24 weeks after ion exchange. In addition, the potential for Ag ion-exchanged glass to cause skin irritation was evaluated using experimental animals, and the cytotoxicity was confirmed. Then, Ag ion elution into drinking water from the ion-exchanged glass was measured to confirm whether the elution of Ag satisfies WHO safety criteria. Finally, the optimum ion exchange condition was confirmed by measuring the transmittance of light and surface roughness of the glass and exploring the relation of these parameters to the penetration depth of the Ag ion. Transparency and a smooth surface are important for application to products such as glassware and display screens.

2. Materials and methods

2.1. Preparation and characterization of Ag ion-exchanged glass

Sheets of aluminosilicate glass 0.7 mm thick (Corning Inc., NY, USA) and composed of 62.4 SiO_2–17.4 Al_2O_3–3.3 MgO–0.2 CaO–0.6 TiO_2–12.6 Na_2O–3.5 K_2O (wt%) were used for ion exchange. High purity (99.8% pure) $AgNO_3$ powder (Dae-Jung Chemicals & Metals Co., Ltd., Pyeongtaek, Korea) was used as the raw material for ion exchange. For the Ag ion-exchange process, the glass substrate was cleaned by sonication in ethanol and deionized water for 10 min to avoid contamination with extraneous organic debris. The glass was then dried in an oven at 110 °C for 2 h. Then, it was placed on a square Al_2O_3 tray, and the $AgNO_3$ powder was spread on both sides of the glass. It was heated in a box furnace (Lindberg/Blue M™, Thermo Scientific, MA, USA) at 250–280 °C for 10 min.

In order to evaluate the penetration depth of Ag and Na ions, measurements were taken from the glass surface to a 30 μm depth using electron probe microanalysis (EPMA, JXA-8900R, JEOL Ltd., Tokyo, Japan). In addition, to precisely determine the amount of silver ions present on the surface of the ion-exchanged glass, the surface of the glass was analyzed by area mapping using a scanning electron microscope (SEM, JSM-6700F, JEOL Ltd.) and energy-dispersive X-ray spectroscopy (EDS) at an acceleration voltage of 20 kV. The average roughness (Ra) and root mean square roughness (Rq) were measured to assess the potential of the ion-exchanged glass for use in display screens by using atomic force microscopy (AFM, XE-100, Park Systems, Suwon, Korea) at the measurement range of 2 μm × 2 μm. The transmittance of visible light was measured using UV/VIS/NIR spectroscopy (V-570, Jasco, Tokyo, Japan) with a 400 nm/min scan speed over the 200–700 nm wavelength range.

2.2. Microbial strains and culture conditions

E. coli ATCC 8739, *S. aureus* subsp. *aureus* ATCC 6538, and *P. funiculosum* ATCC 11797 strains were obtained from the Korean Culture Center of Microorganisms. *E. coli* was grown in nutrient agar medium (Difco 0001: 15 g of agar, 3 g of beef extract, 5 g of peptone, and 1 L of distilled water, pH 7.0). *S. aureus* was cultured in trypticase soy agar medium (BBL 4311768: 15 g of agar, 17 g of pancreatic digest of casein, 3 g of pancreatic digest of soybean meal, 5 g of NaCl, 2.5 g of K_2HPO_4, 2.5 g of glucose, and 1 L of distilled water, pH 7.0). Potato dextrose agar medium (BBL 7149: 15 g of agar, 300 g of diced potatoes, 20 g of glucose, and 1 L of distilled water, pH 5.0) was used to grow *P. funiculosum*.

2.3. Antimicrobial activity

The antimicrobial activity of the ion-exchanged glass was evaluated against *E. coli*, *S. aureus*, and *P. funiculosum* on the basis of the International Organization for Standardization (ISO) method 22196:2011(E). The Ag ion-exchanged glass specimens were prepared as 20 mm × 20 mm squares. They were cleaned by soaking in 70% ethanol in water for 15 min and wiped three times using distilled water due to preventing the elution of Ag components from the glass surface. All experimental equipment was sterilized by autoclaving at 121 °C for 15 min prior to the commencement of experiments. The bacteria and fungi concentrations were confirmed by the dilution plating technique. The cultures were seeded at concentrations ranging from 2×10^5 to 8×10^5 colony forming units (CFU) mL^{-1} in solid medium, and diluted suspensions of each type of bacteria and fungi were obtained. Diluted microbial suspensions, with cell densities of approximately 10^5 CFU mL^{-1}, were transferred (approximately 100 μL $well^{-1}$) to 12-well culture plates containing different types of Ag ion-exchanged and parent (non-ion-exchanged) glass. Then, sterilized film was adhered to both the parent and ion-exchanged glass to stick the bacteria to the glass surface. After 30 min, 1 mL of liquid medium, chosen according to the strain of bacteria/fungi present, was added to each well. Then, 100 μL of mixed liquid medium was spread on solid culture medium. Culture plates seeded with *E. coli* or *S. aureus* were maintained at 37 °C for 24 h in an incubator, while those seeded with *P. funiculosum* were kept at 24 °C for 24 h. After incubation, the viable numbers of *E. coli*, *S. aureus*, and *P. funiculosum* cells were counted. All tests were conducted ten times and the viable bacterial numbers were expressed in log CFU mL^{-1}. The Ag ion-exchanged glass specimens (250–280 °C, 10 min) were evaluated using the ISO 22196 method to assess antimicrobial activity after 1, 4, 12, and 24 weeks. *E. coli*, *S. aureus*, and *P. funiculosum* were seeded on the glass specimens at a concentration of 10^5 CFU mL^{-1}.

2.4. Cell morphology observation using scanning electron microscopy (SEM)

The effect of the silver ions on the surface morphology of microorganisms was confirmed by observing the shape of bacterial (*E. coli* and *S. aureus*) and fungal (*P. funiculosum*) cells grown on the glass before and after ion exchange (260 °C, 10 min). The experimental glass specimen used was stored at room temperature for 24 weeks following completion of the ISO 22196 antimicrobial test at 1, 4, and 12 weeks. After incubation for 24 h at 37 °C (bacteria) or 24 °C (fungi), viable cell numbers were determined. Fixed microbial cells on cleaved mica slides were dehydrated through an ethanol series, from 50% to 100%, critical-point dried, and sputter-coated with a 30-nm thick gold layer. The morphology of microbial cells was analyzed using SEM at an acceleration voltage of 20 kV.

2.5. Cytotoxicity testing of Ag ion-exchanged glass

Cytotoxicity assessment was conducted in accordance with the ISO 10993-5 standards. Prior to the commencement of cytotoxicity testing, all experimental glass specimens, with or without silver, were cleaned by soaking for 15 min in 70% ethanol in water. Glass specimens were then wiped three times using distilled water in order to prevent bacterial contamination. The L-929 (ATCC CCL-1) mouse fibroblast cell line was used in this study and seeded into the wells of a 96-well culture plate (2×10^4 cells mL^{-1}). The extract was prepared in extraction medium (culture medium with fetal calf serum), as it assists with cellular growth. The extract of the parent glass was used as negative control material because it did not produce a cytotoxic response. The $AgNO_3$ powder was used as positive control material because it provided a reproducible cytotoxic response After treatment with Ag ion-exchanged glass, 100 μL of cells were seeded into 96-well plates and incubated at 37 °C/5% CO_2 for 24 h. Cells were washed with phosphate-buffered saline and the extracts were added to the wells (100 μL $well^{-1}$). Cells were then incubated at 37 °C for 24 h, and the extracts were removed from the plates. We added 50 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) solution to each well and incubated the plates for 4 h. The MTT solution was removed and isopropanol (100 μL) was added to each well, and the plates were shaken at 160 rpm for 15 min. Absorption at 570 nm was measured using an ELISA plate reader (Benchmark Plus, Bio-Rad, USA). The relative cell viability was calculated by dividing the absorbance of the specimen by the absorbance of the negative control. Cytotoxicity was classified by ranking, where a score of 0 corresponded to ≥100% viability, 1 corresponded to 75–99% viability, 2 corresponded to 50–74% viability, 3 corresponded to 25–49% viability, 4 corresponded to 1–24% viability, and 5 corresponded to 0% viability. If relative cell viability was over 100% then the material was considered non-cytotoxic.

2.6. Skin irritation testing of Ag ion-exchanged glass

The potential for the Ag ion-exchanged glass to cause skin irritation was evaluated in accordance with the ISO 10993-10 method by conducting experiments at the Korea Testing and Research Institute, based on the guide for the care and use