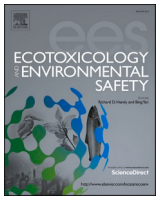
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What do we know about dermal bioaccessibility of metals coated on antibacterial films?

Seon-Woo Kwon [a,1], Junyoung Park [b,c,1], Dong-Jun Baek [d], Haeun Kim [d], Suk Soon Choi [a,e], Jung-Hwan Kwon [f], Jinsung An [d,g,*]

[a] Department of Environment Safety System Engineering, Semyung University, Jecheon 27136, South Korea
[b] Department of Civil and Environmental Engineering, Seoul National University, Seoul 08826, South Korea
[c] Institute of Construction and Environmental Engineering, Seoul National University, Seoul 08826, South Korea
[d] Department of Smart City Engineering, Hanyang University, Ansan 15588, South Korea
[e] Department of Biological and Environmental Engineering, Semyung University, Jecheon 27136, South Korea
[f] Division of Environmental Science and Ecological Engineering, Korea University, Seoul 02841, South Korea
[g] Department of Civil and Environmental Engineering, Hanyang University, Ansan 15588, South Korea

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ABSTRACT

Antibacterial films have gained attention since the outbreak of the COVID-19 pandemic; however, the impact of metals contained in antibacterial films on human safety have not been sufficiently investigated. This study reports on the important features that must be considered when assessing the bioaccessibility of Ag, Cu, and Zn in antibacterial films. Specifically, the effects of the artificial sweat component (i.e., amino acid and pH), surface weathering of antibacterial films, wipe sampling, and sebum were carefully examined. Our findings suggest that amino acids greatly affect bioaccessibility as amino acids act as ligands to facilitate metal ion leaching. In addition, constant exposure to ultraviolet C causes the film surface to oxidize, which significantly increases metal bioaccessibility due to the electrostatic repulsion between metal oxides and organic substrates. The presence of sebum in artificial sweat and physical damage to the film surface had no significant effects. Furthermore, the wipe sampling used to mimic the realistic dermal contact suggests the feasibility of applying this method for the assessment of bioaccessibility of metals in antibacterial films. The method offers significant advantages for evaluating the human safety aspects of skin contact with consumer products in future research.

1. Introduction

Metals from natural and anthropogenic sources cause global environmental problems that adversely impact life outcomes (Bolan et al., 2014; Hassaan et al., 2016; Kapahi and Sachdeva, 2019; Vareda et al., 2019; Dhaliwal et al., 2020; Rajendran et al., 2022). In particular, the widespread use of metal additives in consumer products has been recognized as an exposure route for metals to enter the human body. Consequently, human safety regarding metals contained in consumer products should be comprehensively investigated (Weegels and Van Veen, 2001; López et al., 2002; Basketter et al., 2003; Loretz et al., 2005; Bocca et al., 2014; Arshad et al., 2020). Metals in consumer products can enter the human body mainly through skin contact and ingestion (Yuswir et al., 2013; Mahurpawar, 2015). The skin is the largest organ in the human body and is responsible for various vital functions (body protection, body temperature regulation, balance regulation, and vitamin D synthesis). The substances secreted onto the skin surface are a complicated combination that primarily comprises sweat and sebum (Stefaniak et al., 2010; Marin Villegas and Zagury, 2021). Human sweat contains various substances such as electrolytes, ionic components, organic acids, carbohydrates, amino acids, nitrogen substances, and vitamins, and its composition varies not only between individuals but also according to biological parameters (e.g., age, activity level, gender, and diet) and external features (e.g., environmental, seasonal) (Stefaniak and Harvey, 2006). Lipid components of sebum include squalene, wax esters, triglycerides, free fatty acids, free cholesterol, and vitamin E, and are known to have a slight effect on metal leaching (Stefaniak and Harvey, 2006; Harvey et al., 2010).

* Corresponding author at: Department of Civil and Environmental Engineering, Hanyang University, Ansan 15588, South Korea.
E-mail address: jsan86@hanyang.ac.kr (J. An).
[1] These authors contributed equally to this work.

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Bioaccessibility (migration) refers to the proportion of a substance from the total amount of harmful substances present within confined media that can be absorbed into the human body. It can be determined via appropriate *in vitro* bioaccessibility test methods, which can replace *in vivo* animal assays (i.e., it has high *in vitro—in vivo* correlations) (Cardoso et al., 2015; Xia et al., 2016). *In vitro* test methods for assessing dermal bioaccessibility are based on the principle of extracting metals from consumer products using an artificial sweat solution. Currently, four artificial sweat formulations proposed by the American Association of Textile Chemists and Colorists, International Organization for Standardization, and European Standard are widely used (Kulthong et al., 2010; Li et al., 2021). Among them, the EN 1811 (CEN, 2015) is the most commonly used standard for measuring dermal bioaccessibility of metals, because it is inexpensive, relatively easy to manufacture, and has a high recovery rate in bioaccessibility tests (Pedersen et al., 1974; Lidén et al., 1998; Nygren and Wahlberg, 1998; Bocca et al., 2007). Kulthong et al. (2010) reported that the quantity of Ag released from coated textiles employing these four formulations was maximal in the artificial sweat prepared in accordance with EN 1811. However, this standard has certain limitations, i.e., the absence of amino acids, which have a significant effect on leaching due to interactions with metal ions, and does not account for the effect of sebum (Itoh and Nakayama, 1952; Brown et al., 1982; Flint, 1998; Stefaniak et al., 2014). Furthermore, EN 1811 sets the pH of an artificial sweat solution at 6.5, despite the median pH value of human sweat being approximately 5.3 (Stefaniak and Harvey, 2006). Considering these aspects, amino acids including alanine, glycine, methionine, serine, and threonine must be added to the existing artificial sweat composition of EN 1811 to more closely resemble actual human sweat, the pH must be adjusted to 5.3—5.5, and consideration must be given to the effect of the presence or absence of sebum (Hansen et al., 2003; Van Lierde et al., 2005, 2006).

Although the use of antibacterial films has expanded rapidly due to the recent COVID-19 pandemic, research on the human safety aspects of metals in these products is relatively rare. To address this gap in the literature, this study focused on antibacterial films among other consumer products. Even before the pandemic, the categories of antibacterial products and the market size of antibacterial additives were expanding (e.g., antibacterial masks, disinfectants, detergents, scrubbers, and fabrics), and interest in antibacterial products increased further as a result of the pandemic. Although several studies have investigated the antibacterial properties of Ag, Cu, and Zn ions (Top and Ülkü, 2004; Kubacka et al., 2011; Beigmohammadi et al., 2016; Milenkovic et al., 2017; Poole, 2017; Pemmada et al., 2020; Govind et al., 2021), the current existing literature addressing the risk posed by metals contained in antibacterial films to the human body is limited (Nakashima et al., 2008; Kulthong et al., 2010; Rehman et al., 2018; Yang and Massey, 2019).

The quantity of bioaccessible metals directly influences absorption into organisms, potentially leading to harmful effects. Our investigation centered on antibacterial films as representative samples, chosen due to their ubiquitous presence in public spaces (e.g., elevator buttons) and easy contact with the skin. Specifically, we assessed the amount of metals from antibacterial films that transferred into the human body through skin contact, considering the impact of artificial sweat components (e.g., pH, amino acids, and presence/absence of sebum). Furthermore, we examined changes in bioaccessibility following physical and photochemical weathering to simulate the surface aging of real antibacterial films (Shandilya et al., 2015; Mao et al., 2020; Wang et al., 2020; Aghilinasrollahabadi et al., 2021). Lastly, our study proposed the practicality of wipe sampling, replicating the dermal contact typical in real-world scenarios. This research aimed to provide insights into critical factors that accurately reflect bioaccessibility, aiding the future development of models or assays for evaluating the human health risks associated with commonly used products like antibacterial films.

2. Materials and methods

2.1. Antibacterial film preparation

We procured 11 types of commercially available antibacterial films, all featuring organic substrates (films) doped with Ag, Cu, and Zn nanoparticles (Table 1). To identify the material of each antibacterial film, transmittance was measured in the wavelength range of 500–4000 cm^{-1} with a resolution of 4 cm^{-1} through attenuated total reflectance—Fourier transform infrared spectroscopy (ATR—FTIR, Bruker Lumos, USA).

2.2. Determination of total metal concentrations in antibacterial films

To measure the total metal concentrations in each product, the EPA3052 (USEPA, 1996) was partially modified and used. Cut antibacterial films (0.5 g) were inserted into a Teflon tube containing 10 mL of 65 % HNO_3 (Daejung, Korea), and acid digestion was performed using a microwave device (Mars 6, CEM Corporation, USA) for 20 min at 180 °C with a maximum power of 1200 W. After approximately 20 min, the solution was transferred to a 50-mL conical tube, diluted five times with deionized water, and analyzed via inductively coupled plasma—mass spectrometry (ICP—MS, Agilent Technologies 7900, Japan). To minimize mass interference, ICP—MS equipped with a collision cell was utilized (He flow rate = 5 mL/min) (May and Wiedmeyer, 1998; Collie et al., 2016; Suárez-Oubiña et al., 2022). Samples were analyzed under the following conditions: radio frequency power of 1550 W; radio frequency matching 2 V; and nebulizer gas (Ar) rate of 1.05 L/min. Limits of detection (LODs) were 0.006, 0.133, and 0.059 μg/L for Ag, Cu, and Zn. The analysis result value of the blank (i.e., artificial sweat without antibacterial films) was subtracted from the analysis result value of each sample to correct the matrix effect.

2.3. Determination of dermal bioaccessibility using artificial sweat

Antibacterial films were cut into 10 × 10-cm squares and weighed. The cut antibacterial film specimens and 25 mL of artificial sweat solution were then added to 50-mL polypropylene tubes, allowed to react for 4 h in a water bath at a constant temperature of 37 °C, and filtered through 0.45-μm cellulose acetate filters (Hyundai, Korea). The composition of the artificial sweat solution is detailed in Section 2.3.1.

2.3.1. Effect of pH and presence of amino acids

To evaluate the effect of the artificial sweat solution components on the dermal bioaccessibility of metals in antibacterial films, two artificial sweat solutions were prepared and tested. Sweat A, suggested by Van

Table 1
Total metal concentrations in antibacterial films used in this study.

Sample ID	Material[a]	Total concentration (mg/kg)		
		Ag	Cu	Zn
F-1	PVC	4.01 ± 0.492[b]	1540 ± 28.2	740 ± 1.62
F-2	PE	-[c]	1690 ± 8.18	743 ± 0.283
F-3	PVC	3.46 ± 0.433	-	353 ± 2.67
F-4	PET	-	1010 ± 7.40	17.5 ± 3.30
F-5	PVC	3.76 ± 0.465	-	352 ± 13.1
F-6	PVC	4.38 ± 0.359	-	352 ± 9.16
F-7	PE	-	84.4 ± 11.6	10.8 ± 3.71
F-8	PE	-	-	86.5 ± 9.62
F-9	PE	-	-	1040 ± 66.0
F-10	PP	1.56 ± 0.00771	-	103 ± 0.0777
F-11	PP	5.43 ± 1.12	-	464 ± 3.59

[a] PVC = polyvinyl chloride, PE = polyethylene, PET = polyethylene terephthalate, PP = polypropylene.
[b] '±' indicates the standard deviation of triplicates (n = 3).
[c] '-' indicates that the total metal concentration was below the detection limit (Ag: 0.0006 mg/kg, Cu: 0.013 mg/kg, and Zn: 0.006 mg/kg).

Lierde et al. (2005), contained five amino acids (alanine, glycine, methionine, serine, and threonine), and had a pH of 5.5. Sweat B, which was prepared according to the EN 1811 (CEN, 2015), did not contain amino acids and had a pH of 6.5. Specifically, Sweat B was prepared by adding NaCl (5 g), lactic acid (0.94 mL), and urea (1 g) to 900 mL of deionized water. After titrating the pH to 6.5 using 0.1 M NaOH and HCl, the volume was adjusted to 1 L by adding deionized water. Sweat A was prepared by adding 1 g/L of each of the five amino acids to Sweat B and titrating the solution to a pH of 5.5.

2.4. Effect of surface weathering of antibacterial films on dermal metal bioaccessibility

Long-term usage of antibacterial films in the real world may cause surface abrasion. A physical weathering test (i.e., reciprocating wear test) and a photochemical weathering test (i.e., UV irradiation test) were performed to assess the effect of surface aging generated through external exposure on the dermal bioaccessibility of metals. To simulate physical weathering caused via repetitive contact with antibacterial films, a rubbing tester was subjected to the test conditions of a 500-g load, reciprocating speed of 60 rpm, and sandpaper abrasion (NR-RB1, NURISC, USA). By referring to the range of the Martindale technique used to measure the abrasion resistance of textiles, the number of rubbings was set to 2500, 5000, 10,000, 20,000, and 40,000 cycles (Bilisik and Yolacan, 2009; ISO, 2016; Siddika et al., 2017). To simulate photochemical weathering, the antibacterial films were exposed for 1, 2, 4, 7, 14, and 28 d in a steel container fitted with four 15-W ultraviolet C (UVC) lamps (Sankyo Denki, Japan). ATR—FTIR and X-ray photoelectron spectroscopy (XPS) (K-ALPHA, Thermo Fisher Scientific, UK) were used to observe changes in the surface properties of antibacterial films that occurred after weathering.

2.5. Wipe sampling

The applicability of the wipe sampling method, which simulates contact between the sample and the skin more realistically, was reviewed. Briefly, the sample preparation of the wipe sampling method followed that of Brookhaven National Laboratory (2014), the surface wiping process was performed in an S pattern (Fig. S1) on the basis of the NIOSH guideline Elements on Wipes: Method 9102 (NIOSH, 2003), and the analysis procedure followed the method of Mackevica et al. (2018). The wipe sampling method was prepared by cutting a wipe (WW-2109, non-woven fabric, Korea) into a size of approximately 5 × 5 cm and adding 0.5 mL of artificial sweat. The process of wiping the entire surface of the antibacterial film in an S pattern was repeated three times while holding the prepared wipe with two fingertips with a pressure of 462 g/cm^2, which is similar to the force exerted by a person sitting on a chair (Clausen et al., 2016).

As shown in Fig. S1, each time the entire surface was wiped, the exposed side of the wipe was folded in half, and the same surface was wiped in a different direction. After the wiping process, the wipe was placed in a 50-mL polypropylene tube with 20 mL deionized water and sonicated for 10 min in a sonicator (NXPC-B3010S, Komachine, Korea). The wipe was then removed using a tweezer, and the solution was filtered through a 0.45-μm cellulose acetate filter (Hyundai, Korea). The solution was further analyzed via ICP-MS and converted into the available mass to calculate the bioaccessible concentration of metal out of the total mass of the antibacterial film. The wipe sampling method was replicated three times, and the mean concentration was reported with error bars of standard deviations.

2.6. Effect of presence of sebum

To examine the effect of the presence of sebum on the dermal bioaccessibility of metals, two types of artificial sebum were prepared and tested. Sebum A comprised seven lipids including squalene, lanolin, tristearin (saturated triglycerides), triolein (unsaturated triglycerides), palmitic acid (saturated free fatty acids), oleic acid (unsaturated free fatty acids), and cholesterol) at concentrations of 10.49, 24.75, 21.79, 10.90, 14.05, 14.05, and 3.97 % w/w, respectively, which are major constituents of sebum (Stefaniak and Harvey, 2006). Notably, Sebum B, containing vitamin E (1.0 % w/w), had a simpler composition than Sebum A (squalene, jojoba oil, triolein, and oleic acid at 12.4, 25, 44.6, and 17.0 % w/w, respectively) (Wertz, 2009). The components of Sebum A and B were added to a solution prepared by mixing chloroform and methanol in a volume ratio of 2:1. The mixture was heated to a temperature of $\geq$ 45 °C and stirred until all components were leached. Thereafter, 25 mL of the sebum—component mixture solution was placed in a glass petri dish, and a 47-mm paper filter (KFP, Korea) was inserted. The solvent was evaporated to coat the paper filter surface with sebum components (Stefaniak and Harvey, 2008). The prepared sebum was then administered in the dermal bioaccessibility test using artificial sweat, and the change in the leached quantities of metals in the antibacterial films according to the presence or absence of sebum was observed.

3. Results and discussion

3.1. Total and bioaccessible metal concentrations in antibacterial films

Ag and Cu have been studied as antimicrobial agents, and ZnO nanoparticles have been shown to play a significant role in facilitating or maintaining antimicrobial activity (Piedade et al., 2020; Ermini and Voliani, 2021; Yoon et al., 2022). Although the specific antibacterial mechanism is unclear, studies suggest that metals induce oxidative stress by generating reactive oxidant species, which may lead to cell apoptosis or directly damage nucleic acids (i.e., DNA and RNA) (Ermini and Voliani, 2021). Above all, the metal forms and species were found to be critical, where oxidation levels of +1 showed superior antiviral activity compared to that of the other states (Yoon et al., 2022). In this regard, semi-conducting materials, such as ZnO, are often chosen to produce alloys with antimicrobial agents to maintain their activities by transferring electron to metals to maintain their efficient oxidation states due to the photocatalytic properties under light irradiation. Therefore, Ag, Cu, and Zn were selected as target metals to assess bioaccessibility in this study. Specifically, total metal concentrations in 11 types of antibacterial films (F-1 to F-11) were measured (Table 1). The concentrations of Ag, Cu, and Zn were < 0.0006 (below the detection limit [BDL]) to 5.43 ± 1.12 mg/kg, < 0.013 (BDL) to 1690 ± 8.18 mg/kg, and 10.8 ± 3.71–1040 ± 66.0 mg/kg, respectively. Zn was present in all the films. In the case of Ag, its higher cost may have been the reason for its lower concentrations in antibacterial films compared to those of the other metals (Roy et al., 2016).

In vitro bioaccessibility tests for dermal exposure with two types of artificial sweat (A: amino acids + pH 5.5; B: pH 6.5) were utilized to elute metals from 11 different antibacterial films. Overall, the leached amounts of Cu and Zn, which were directly related to the bioaccessible concentrations, were higher when eluted via Sweat A (Table 2), whereas Ag was not identified because the total concentration was low. For example, the leaching test indicated a 4-fold higher Cu concentration in Sweat A (0.66 ± 0.04 mg/kg) than in Sweat B (0.15 ± 0.02 mg/kg) in the F-1 sample. Presumably, this trend was attributed to enhanced metal mobility owing to the decreased pH (Stefaniak and Harvey, 2006) and the addition of amino acids, which influence metal leaching (Flint, 1998; Van Lierde et al., 2005). Particularly, amino acids are known to either chelate or act as ligands to form complexes via coordinate bonding and conjugation (Kaewchangwat et al., 2017; Badetti et al., 2019). The solution medium should be alkaline to form chelates between Cu ions and deprotonated carboxylic groups along with amino groups. Direct chelation with Cu ions is unlikely to have occurred as our media of artificial Sweat A had a pH of 5.5, yielding a protonated form of amino groups where the pKa values were ~9, and H^+ interference was inevitable.

Table 2
Bioaccessible metal concentrations in antibacterial film (mg/kg ± standard deviation) for dermal exposure determined using two artificial sweats and their calculated bioaccessibility (values in parentheses).

Film	Sweat A (pH 5.5 + amino acids)			Sweat B (pH 6.5)		
	Ag	Cu	Zn	Ag	Cu	Zn
F-1	-[a]	0.665 ± 0.0432 (0.04 %)	2.95 ± 0.228 (0.40 %)	-	0.148 ± 0.0240 (0.01 %)	2.18 ± 0.189 (0.29 %)
F-2	-	5.80 ± 0.681 (0.34 %)	3.80 ± 0.0915 (0.51 %)	-	3.24 ± 0.820 (0.19 %)	1.87 ± 0.0570 (0.25 %)
F-3	-	-	1.44 ± 0.160 (0.41 %)	-	-	0.658 ± 0.0465 (0.19 %)
F-4	-	40.5 ± 3.16 (4.01 %)	0.468 ± 0.0237 (2.69 %)	-	21.1 ± 2.63 (2.09 %)	0.176 ± 0.0894 (1.03 %)
F-5	-	-	1.89 ± 0.186 (0.54 %)	-	-	1.04 ± 0.109 (0.30 %)
F-6	-	-	1.31 ± 0.189 (0.37 %)	-	-	0.629 ± 0.123 (0.18 %)
F-7	-	22.1 ± 1.35 (26.17 %)	0.327 ± 0.0554 (3.06 %)	-	11.4 ± 0.636 (13.78 %)	0.161 ± 0.0692 (1.48 %)
F-8	-	-	1.51 ± 0.0697 (1.75 %)	-	-	0.380 ± 0.0630 (0.44 %)
F-9	-	-	19.2 ± 0.348 (1.84 %)	-	-	18.0 ± 1.81 (1.73 %)
F-10	-	-	52.8 ± 0.121 (51.35 %)	-	-	59.9 ± 2.91 (58.29 %)
F-11	-	-	107 ± 1.39 (22.93 %)	-	-	42.5 ± 3.84 (9.16 %)

[a] '-' indicates that the bioaccessible metal concentration was below the detection limit (Ag: 0.0001 mg/kg, Cu: 0.0017 mg/kg, and Zn: 0.0007 mg/kg).

Instead, we posit that the leaching process occurs because of the conjugation of metals by amino acids. This inference is supported by the findings of Badetti et al. (2019), who observed that the complexation between CuO and amino acids led to the ligand-assisted leaching of Cu ions. Similarly, our result showed that relatively high levels of metals were eluted in the presence of amino acids when comparing the case where amino acids are not present in sweat. This phenomenon may be attributed to the interaction between hydroxyl groups on the surfaces of CuO and ZnO with the carboxylic and amino groups of amino acids, leading to ligation (chelation) triggered by hydrogen bonding. In addition, the influence of electrostatic forces contributed to the removal of metal ions from the solid surfaces, suggesting the susceptibility of films to leaching (Mills et al., 2021).

Furthermore, no proportionate relationship could be identified between total metal concentrations and bioaccessibility (Tables 1 and 2), or between the antibacterial film material and bioaccessibility. We hypothesized that diverse physical forms (i.e., oxide vs. ion) and chemical states (i.e., oxidation state) present in different proportions in antibacterial films may lead to varying dissolution behaviors because of complexation with ligands (as observed with Sweat A) or leaching (as observed with Sweat B). Yoon et al. (2022) indicated that the degree of leached Cu ions varied depending on the specific forms of metals. Moreover, the lack of correlation emphasizes the importance of prioritizing measurements of bioaccessible levels in health risk assessment, as the leached levels are directly associated with bioavailability rather than the total concentration of metal contents. Ultimately, the present study demonstrated that the experimental (*in vitro*) assessment of bioaccessibility should consider pH, amino acids, and metal form when developing models or assays to evaluate human health risks related to dermal exposure to metal-containing products, including antibacterial films.

3.2. Effect of surface weathering of antibacterial films on dermal metal bioaccessibility

Physical weathering (surface rubbing test) and photochemical weathering (UVC irradiation test) were performed on F-7, which possessed a relatively high bioaccessible Cu concentration. Fig. 1a shows that even as the number of rubbings increased, the bioaccessible Cu concentration did not change significantly. However, with an increasing period of UVC exposure, the bioaccessible Cu concentration increased modestly up to 1.5 times (Fig. 1b). ATR—FTIR (Fig. 2) and XPS analyses on the surface of the photochemically weathered antibacterial film were performed to assess the causes of bioaccessibility changes. A significant increase in the ATR—FTIR transmittance pattern (Fig. 2) was observed following UVC exposure at wavenumbers of 1218, 1014, and 966 cm^{-1}, indicating the further occurrence of C-O bonding structures (Wang et al., 2020; Doğan, 2021). Furthermore, results of XPS analysis indicate an increase in the O/C ratio after the UVC exposure (C: 57.2 %, O: 35.8 %, N: 2.4 %, Si: 4.2 %, O/C ratio: 0.63) compared to that before the exposure (C: 65.8 %, O: 30.6 %, N: 1.1 %, Si: 2.4 %, O/C ratio: 0.47). To further verify the oxidation process in carbon, XPS carbon spectra (specifically, C1s) were fitted and subjected to deconvolution. Figure S2 indicates the formation of C-O and C═O after UVC exposure accompanied by changes in atomic percentages of C-C/C-H, C═O, and C-O from 64.8, 14.3, and 20.9 % to 47.3, 19.8, and 32.9 %, respectively. Taken together, these results demonstrated the occurrence of surface oxidation of antibacterial films (Stark and Matuana, 2004; Gardette et al., 2013; Fritz et al., 2022).

Fig. 3 presents the surface images of film F-7 before and after the rubbing and UVC irradiation tests analyzed using scanning electron microscopy (SEM). Physical weathering of the surface of antibacterial films caused serious damage to the film surface, altering its morphology; however, frequent contact did not result in substantial changes in metal bioaccessibility, which was contrary to expectations. However, it was evident that the surface oxidation of antibacterial films occurred during long-term UV exposure, which demonstrably increased metal bioaccessibility. In particular, UV oxidized the organic substrate to increase the O concentration, resulting in the generation of ionizable functional groups and the development of a negative surface charge in addition to changing the morphological structure. Konkena and Vasudevan (2012) similarly observed that the graphene oxide had a lower zeta potential than the reduced graphene oxide due to oxygen-based functional groups. These results revealed that morphological changes did not result in increased Cu leaching; rather, chemical properties, particularly surface charges brought on by functional groups, critically affected bioaccessibility. In turn, the films would possess a negative surface charge, resulting in electrostatic repulsion between metal oxides, and the film substrate may eventually provide a suitable environment for easy leaching when the films are immersed in a pH 5.5 solution, and the zeta potential of CuO is negative (El-Trass et al., 2012). Notably, sample preparation considering the photochemical damage of the antibacterial film surface is necessary for a realistic assessment of dermal metal bioaccessibility.

3.3. Wipe sampling

Our study identified sweat containing amino acids (Sweat A) as a significant factor in metal leaching, prompting questions about the adequacy of bioaccessibility assessment methods for measuring metal levels in sweat solutions. Specifically, we aimed to explore a more realistic approach to accurately assess metal bioaccessibility, termed 'wipe sampling.' As outlined in Section 3.2, frequent contact with antibacterial films in public spaces suggests potential dermal exposure, motivating our focus on elucidating the bioaccessibility of Cu and Zn

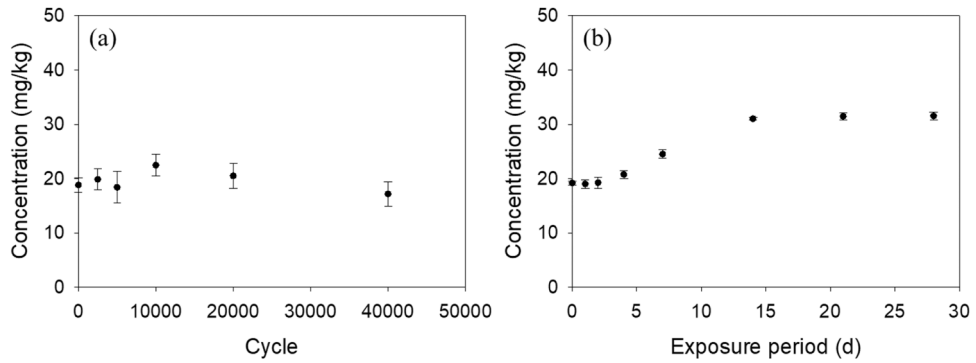


Fig. 1. Dermal bioaccessible Cu concentration in the antibacterial film F-7: (a) physical weathering (i.e., rubbing test); (b) photochemical weathering (i.e., ultraviolet C [UVC] irradiation test). Error bars represent the standard deviations of experimental triplicates.

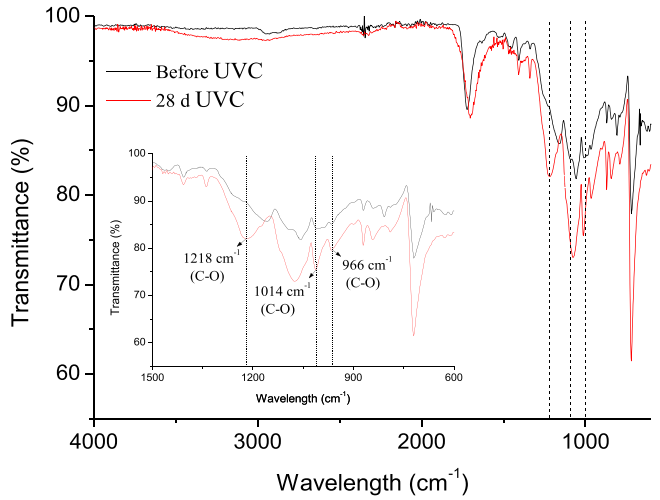


Fig. 2. Results of Fourier transform—infrared (FT—IR) analysis for the antibacterial film F-7 before and after ultraviolet C (UVC) irradiation.

(note: Ag was excluded due to its low levels) through wiping the antibacterial film surface with nonwoven fabric moistened with a small amount of artificial Sweat A or B to mimic fingertip dermal contact. As depicted in Fig. 4, bioaccessible levels varied between the leaching test (F-4 Cu: 40.51 ± 3.16 mg/kg, F-11 Zn: 106.51 ± 1.39 mg/kg) and wipe sampling (F-4 Cu: 0.16 mg/kg ± 0.02, F-11 Zn: 2.40 ± 0.53). Notably, the leaching test indicated Cu concentrations of up to 200 times and Zn concentrations of up to 40 times higher than those determined by wipe sampling. This discrepancy was expected, given the use of lower volumes of artificial sweat (25 mL for leaching test and 0.5 mL for wipe sampling) and a considerably shorter incubation time in wipe sampling. Further insights into differences in migrated amounts due to incubation time (0—96 h) were provided by the leaching test (Fig. S3). Cu and Zn concentrations reached equilibrium, saturating the fabric within 24 h, indicating that metal migration was influenced by contact time. However, contact with antibacterial films for > 24 h is uncommon in realistic scenarios, suggesting that time would have a negligible impact, with deviations of migrated levels within 2.08 % for Cu and 6.93 % for Zn during the first 2 h. Notably, wipe sampling demonstrated consistency with the leaching test regarding the proportions of Cu and Zn leached; specifically, dermal migration values for Sweat A were higher than those for Sweat B, mirroring the leaching test results. Therefore, the inclusion of wipe sampling appears feasible for determining leached levels and reflecting realistic bioaccessibility.

3.4. *Effect of presence of sebum*

The change in the bioaccessible metal concentrations according to the presence of artificial sebum was examined with three antibacterial films (F-2, F-4, and F-7). Human skin contains a substantial quantity of sebum; thus, the effect of sebum components on metal concentrations leached from antibacterial films by sweat was examined. The presence of Sebum A or B did not exert a significant effect on the dermal bioaccessibility of Cu or Zn (Fig. 5), as indicated by non-significant differences in most of the leached levels (p values of 0.078–0.973) according to analysis of variance tests. An exception was observed for Cu and Zn levels in Sebum A, with p values of 0.004 and 0.039, respectively, suggesting statistical significance. However, in terms of deviation, the observed differences in levels did not exceed 15 %, indicating subtle variations. In essence, considering the simplicity of model development, the incorporation of artificial sebum may not be necessary in *in vitro* dermal metal bioaccessibility tests for antibacterial films because the addition of hydrophobicity contributed minimally, if at all, to metal leaching.

4. Conclusions

This study investigated the impact of artificial sweat solution

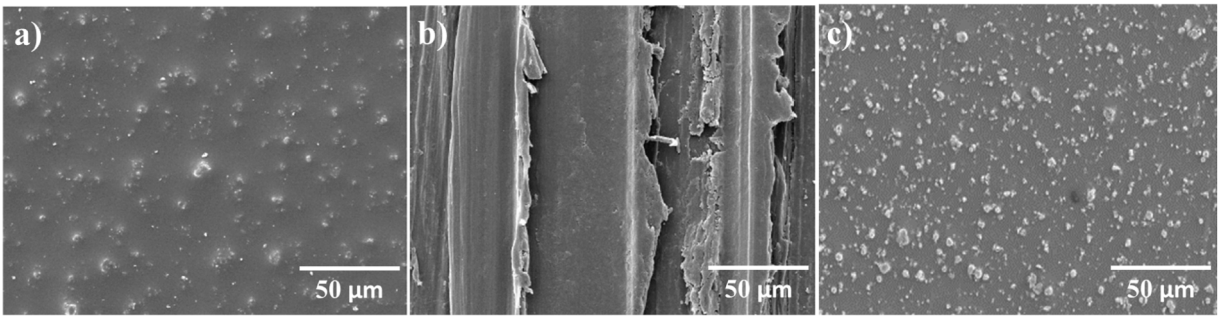


Fig. 3. Scanning electron microscopy (SEM) images of (a) raw film F-7, (b) film F-7 after rubbing test, and (c) film F-7 after UVC irradiation test.

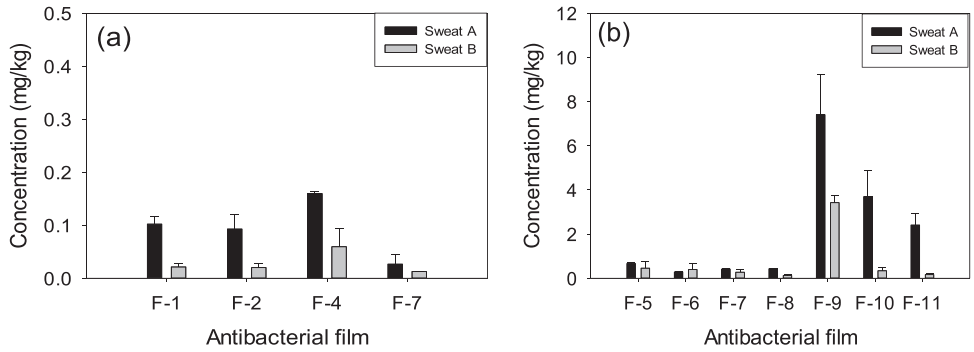


Fig. 4. Dermal bioaccessible concentration of (a) Cu and (b) Zn in antibacterial films after wipe sampling. The films were wiped three times in an S pattern with a pressure of 462 g/cm^2. Error bars represent the standard deviations of experimental triplicates.

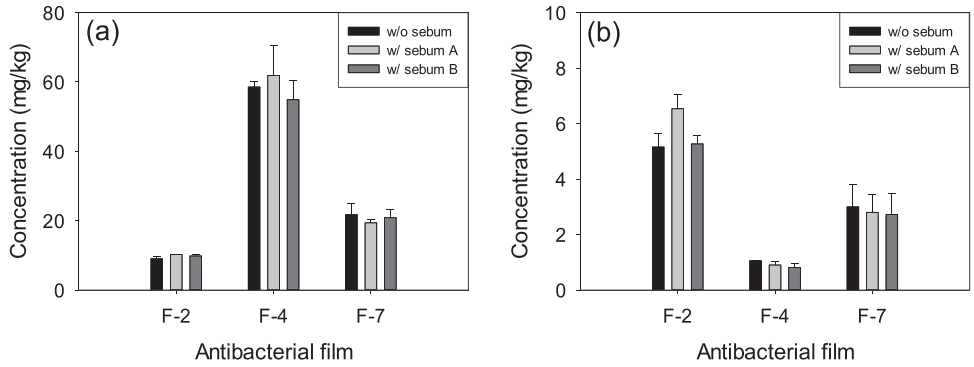


Fig. 5. Dermal bioaccessible concentration of (a) Cu and (b) Zn in antibacterial films (F-2, F-4, and F-7) with and without the addition of artificial Sebum A or Sebum B. Error bars represent the standard deviations of experimental triplicates.

composition, film surface weathering, wipe sampling, and the presence or absence of sebum on the dermal bioaccessibility of Ag, Cu, and Zn in 11 antibacterial films. When utilizing Sweat A, which closely resembled actual human sweat in composition and pH, metal bioaccessibility was observed to be up to four times higher compared to using Sweat B. This suggests that amino acids play a crucial role in facilitating metal leaching via ligand-assisted interaction, thus contributing significantly to metal bioaccessibility. Additionally, neither the presence of sebum in artificial sweat nor physical surface deterioration from repeated contact had any discernible effects on the metal bioaccessibility of antibacterial films. Changes in morphology and hydrophobicity were not deemed significant features to consider. However, the oxidation of the film surface due to constant UVC exposure notably increased metal bioaccessibility. This increase was attributed to the negative surface charges causing electrostatic repulsion between metal oxides and the organic substrate, possibly generated by O-based functional groups. Furthermore, the feasibility of wipe sampling, which mimicked dermal contact, was suggested as a method to assess the bioaccessibility of Cu and Zn, leading to a more realistic human health safety assessment. While our findings indicated low bioaccessible metal levels, typically ranging from nanograms to milligrams per kilogram of films—far below lethal doses (e.g., 10–20 g for Cu)—concerns regarding chronic toxicities from bioaccumulation in the blood due to frequent contact, especially considering the widespread use of antibacterial films post-pandemic, remain (Gray et al., 2017). Ongoing studies are focusing on the migration and permeation of metals from the skin into the epithelial cell layer (blood vessels) to gain a deeper mechanistic understanding of the uptake processes. Based on our findings thus far, an *in vitro* model for assessing dermal bioaccessibility is briefly suggested in Fig. 6, considering important features such as amino acids and pH, as well as realistic scenarios such as photochemical weathering and wipe sampling. Our study provides insights for further model development by incorporating features that significantly affect metal bioaccessibility.

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CRediT authorship contribution statement

Seon-Woo Kwon: Writing – original draft, Methodology, Investigation. **Junyoung Park:** Writing – original draft, Investigation. **Dong-Jun Baek:** Investigation. **Haeun Kim:** Investigation. **Suk Soon Choi:** Writing – review & editing. **Jung-Hwan Kwon:** Funding acquisition, Conceptualization. **Jinsung An:** Writing – review & editing, Supervision, Resources, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Data availability

Data will be made available on request.

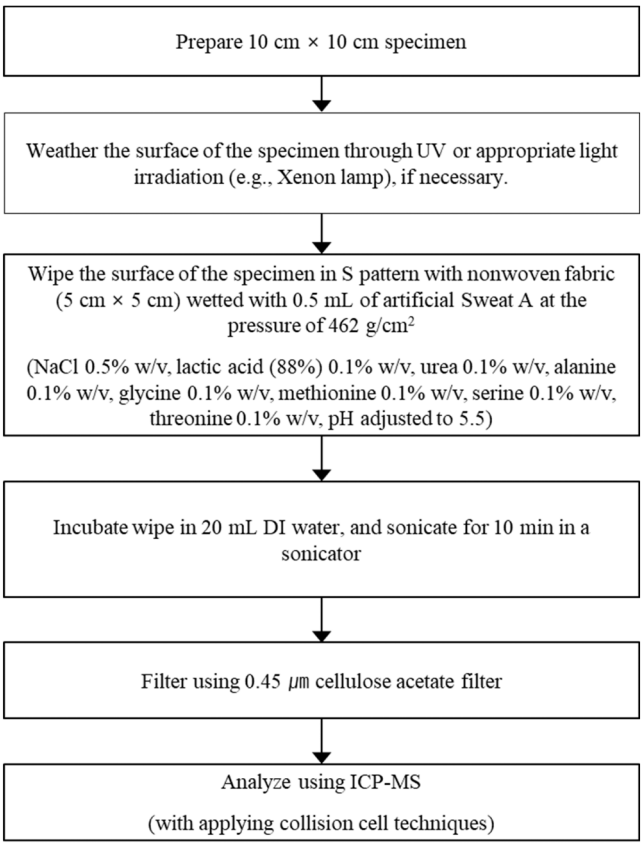


Fig. 6. Suggested *in vitro* bioaccessibility test method for metals contained in consumer products in the form of film proposed in this study. UV – ultraviolet, DI – deionized, ICP-MS – inductively coupled plasma mass spectrometry.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.ecoenv.2024.116213.

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