Quantification of aluminium release from Finn chambers under different in vitro test conditions of relevance for patch testing

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Abstract

Background: Contact allergy to aluminium (Al) might pose a risk of false-positive readings of patch-test results when testing with Finn chambers.

Objectives: To quantify the release of Al from empty Al Finn chambers, covered Finn Aqua chambers, and Al Finn chambers containing different baseline patch-test substances.

Methods: Al Finn chambers of different conditions and with different patch-test substances were tested in artificial sweat and their Al release was analyzed by atomic absorption spectroscopy and inductively coupled plasma sector field mass spectrometry.

Results: The amount of Al released from empty Finn chambers corresponded to a skin dose of 0.03%–0.5% Al chloride hexahydrate applied in plastic chambers. Although most patch-test substances reduced the release of Al from the Finn chambers due to covering the surface, some substances significantly increased the release of Al from the Finn chambers, most notable for Caine mix II 10% pet., Myroxylon per- eirae 25% pet., and sodium tetrachloropalladate hydrate 3.0% pet.

Conclusions: The release of Al from Finn chambers corresponds in some cases to clinically relevant concentrations of Al for Al-sensitized individuals.

KEYWORDS
allergic contact dermatitis, aluminium, patch testing

1 | INTRODUCTION

Contact allergy to aluminium (Al) among dermatitis patients in general has not often been reported, and as this substance is not included in the baseline series in most countries, the real frequency of this contact allergy is unknown. In a recent French study,1 a surprisingly high frequency of contact allergy to Al (21.6%) was reported in consecutively patch-tested children. Vaccines and immunotherapy seem to be main causes of the development of contact allergy to Al2-6; however, a recent study could not confirm the clear role of immunotherapy.2 In a Swedish prospective cohort study comprising 4758 children, 0.83% of vaccinated children developed intensely itching subcutaneous nodules at the injection site for Al-adsorbed vaccines.7 Generally, the higher the Al dose and the more frequently injections are given, the higher the risk for developing contact allergy to Al2,7; individuals with atopic dermatitis seem to have an increased risk.2 Once sensitized, other elicitation sources can be cosmetics, deodorants, Al metal, eardrops, toothpaste, and tattoos,9 but the bioavailability of different Al sources is not well investigated. A recent Danish questionnaire study of 177 Al-allergic children and their parents compared with a reference group concluded that itching vaccination granulomas and Al allergy have a considerable negative impact on these children and their families, causing for instance, reduced adherence.
to vaccination programs and a lower score on overall life quality as compared to the reference group. Contact allergy to Al is not easily diagnosed, as the elicitation threshold might be higher than the patch test substance used and since there is a considerable individual over-time variation in patch-test results, resulting in a high risk of false-negative results in Al-allergic individuals. A recent study on 241 children with previous vaccine-induced itching nodules found that patch testing with 2% Al chloride hexahydrate in pet. gave more positive reactions as compared with an empty Al Finn chamber. It has been reported, however, that false-positive reactions to various allergens applied in Finn chambers can occur in Al-allergic individuals.

The objectives of this study were to (a) quantify the release of Al from Al Finn chambers and Finn chambers Aqua (covered Al chambers) as compared to common patch-test skin doses of Al chloride hexahydrate and (b) to quantify the release of Al from Al Finn chambers containing different baseline patch-test substances.

2 | MATERIALS AND METHODS

2.1 | Al release testing

The Al release from Finn chambers (about 0.05 g weight) and Finn chambers Aqua was tested in vitro in artificial sweat (ASW, 5.0 g/L NaCl, 1.0 g/L urea, 1.0 g/L lactic acid, pH adjusted to 6.5 ± 0.05 with NaOH). All chemicals for artificial sweat were of analytical grade and obtained from VWR, Sweden. The total surface area of the Finn chambers (both sides) was approximately 1.57 cm² (their outer diameter was 10 mm) and the total volume of the ASW was 1.57 mL, giving a surface area to volume ratio of 1 cm²/mL. The Finn chambers Aqua (Sample only, Lot# 1618GEN, expiry December 31, 2020, SmartPractice, Phoenix, Arizona), and their corresponding reference samples (batch 2 Finn chambers, on Scanpor, Sample only, Lot# 1618GEN, expiry December 31, 2022, SmartPractice), were sealed with a metal-free lacquer (Nail Polish, Coverage, Depend O2, Nr. 033, Lot nr. 37 855, Depend Cosmetic, Halmstad, Sweden) on the back side, so that only the paper-covered front side (0.785 cm²) was exposed to the artificial sweat (1.57 mL), corresponding to a surface area to volume ratio of 0.5 cm²/mL. Finn chambers of different types and after different surface and cleaning conditions were compared (Table 1). In addition, Al Finn chambers (batch 3) with applied patch-test substances were tested (Tables 1-2). Thirty-two different patch-test substances (Table 2) were applied on 2–3 Finn chambers for each substance, with 15 μL for aqueous solutions and 20 mg for substances in pet., which is in agreement with European guidelines for patch testing in 8 mm (inner diameter) Finn chambers. For the application of pet. substances, the Finn chamber was placed on a balance (0.1 mg accuracy) and 20 mg of the pet. substance was added by means of a cleaned stainless steel spoon onto the Finn chamber, covering an area of 0.3–0.5 cm². For application of the aqueous solution, a round (0.5 cm diameter) paper filter (Finn Chambers Filter Paper Discs, Lot# ALBAGIRM, SmartPractice) was placed on the Finn chamber and 15 μL of the aqueous patch-test substance was added onto that paper filter. The Finn chamber with the patch-test substance was then placed in an acid-cleaned (10 vol% nitric acid for at least 24 hours followed by four times rinsing with ultrapure water) centrifuge tube and the ASW was added. Due to the conical shape of the centrifuge tube, the entire Finn chamber was exposed to the ASW solution. Batch 1 and batch 3 Finn chambers (SmartPractice) have been obtained from a patch-test clinic (Centrum of Occupational and Environmental Medicine, Stockholm) with unknown storage history of at least some months. Batch 2 and the Finn chambers Aqua have been obtained as samples from SmartPractice participating at the Congress of the European Society of Contact

| ID/name/number (n) | Type of chambers | Surface preparation | Cleaning | Exposure |
|-------------------|------------------|---------------------|----------|----------|
| "Not cleaned," n = 1 | Al Finn chambers, batch 1 | - | - | Ultrapure water (MQ), 48 h, 30 °C |
| "Not cleaned," n = 3 | - | - | - | Artificial sweat (ASW), 48 h, 30 °C, no agitation |
| "Cleaned," n = 3 | - | 5 min in ethanol and subsequently 5 min in acetone in an ultrasonic bath, dried with nitrogen gas at room temperature |
| "Ground + cleaned," n = 3 | Finn chambers Aqua | An area of about 0.25 of 1.57 cm² ground with 1200 grit SiC paper and water, about 30 min prior to immersion |
| "Aqua," n = 3 | Finn chambers Aqua | - | - | |
| "Al Finn" (control for Aqua), n = 3 | Al Finn chambers, batch 2 | - | - | |

Three background (blank) samples for batch 1 and two blank samples for batch 2 without any Finn chamber in artificial sweat were also measured.
Dermatitis in October 2018, with most probably the shortest time of storage. The two palladium patch-test substances have been included for the study, since they (a) were included in the extended baseline series at the Centrum of Occupational and Environmental Medicine, Stockholm, (b) contained high amounts of chlorides, which are known to cause Al corrosion,\(^{15}\) and (c) due to observed cases at the Centre of Occupational and Environmental Medicine, Stockholm, where Al-allergic patients reacted positive to palladium test substances when tested with Finn chamber but negative in IQ-chamber. This fact could be explained (among others) by false-positive reaction due to Al release.

All specimens (with Finn chambers) and blank samples (without any Finn chambers) were exposed for 48 hours at 30 ± 0.5°C with no agitation. All conditions are according to the EN 1811:2011 + A1:2015 standard,\(^{13}\) except the immersion time, which was adjusted to the 48 hours of normal patch testing. Prior to the immersion and after immersion, the pH was measured of all samples. The pH values

| Patch-test substance and concentration (reference) | Number | Number of replicates (n) |
|---------------------------------------------------|--------|--------------------------|
| [Tested patch-test substances for Al release study from Finn chambers (batch 3)](https://doi.org/10.1002/cnd.24322) |
| (reference)                                        | -      | 5                        |
| (reference with paper filter)                     | -      | 4                        |
| Potassium dichromate 0.5% pet.                    | 1      | 3                        |
| p-Phenylenediamine (PPD) 1% pet.                  | 2      | 3                        |
| Thiuram mix 1% pet.                               | 3      | 3                        |
| Neomycin sulfate 20% pet.                         | 4      | 2                        |
| Cobalt(II) chloride hexahydrate 1% pet.           | 5      | 3                        |
| Quaternium-15 1% pet.                             | 6      | 2                        |
| Nickel(II) sulfate hexahydrate 5% pet.            | 7      | 3                        |
| Quinoline mix 6% pet.                             | 8      | 3                        |
| Colophonium 20% pet.                              | 9      | 2                        |
| Paraben mix 16% pet.                              | 10     | 3                        |
| Black rubber mix 0.6% pet.                        | 11     | 2                        |
| Sesquiterpene lactone mix 0.1% pet.              | 12     | 3                        |
| Mercapto mix 2% pet.                              | 13     | 3                        |
| Epoxy resin (diglycidyl ether of bisphenol A) 1% pet. | 14     | 2                        |
| Myroxylon pereirae (balsam of Peru) 25% pet.      | 15     | 3                        |
| p-tert-Butylphenolformaldehyde resin 1% pet.      | 16     | 3                        |
| Fragrance mix II 14% pet.                         | 17     | 3                        |
| Formaldehyde 2% aq.\(^{a}\)                       | 18     | 3                        |
| Fragrance mix I 8% pet.                           | 19     | 3                        |
| Phenol formaldehyde resin 1% pet.                 | 20     | 3                        |
| Diazolidinyl urea 2% aq.\(^{a}\)                  | 21     | 3                        |
| Methylchloroisothiazolinone/ methylisothiazolinone0.02% aq.\(^{a}\) | 22     | 3                        |
| Amerchol L-101 50% pet.                           | 23     | 3                        |
| Caine mix II 10% pet.                             | 24     | 3                        |
| Lichen acid mix 0.3% pet.                         | 25     | 3                        |
| Tixocortol-21-pivalate 0.1% pet.                  | 26     | 3                        |
| Textile dye mix 6.6% pet.                         | 27     | 3                        |
| Budesonide 0.01% pet.                             | 28     | 3                        |
| Methylidibromo glutaronitirile 0.5% pet.          | 29     | 3                        |
| Methylisothiazolinone 0.2% aq.\(^{a}\)            | 30     | 3                        |
| Palladium chloride 2% pet.                        | 31     | 3                        |
| Sodium tetrachloropalladate hydrate 3% pet.       | 32     | 3                        |

Surface conditions for Finn chambers: As-received (not cleaned, not ground). Five background (blank) samples without any Finn chamber were also measured. Exposure conditions: Artificial sweat (ASW), 48 hours, 30°C, no agitation.

Abbreviations: aq., in water; pet., in petrolatum.

\(^{a}\)Aqueous substances were applied to a Finn chamber containing a paper filter.
decreased during immersion from pH 6.5 to pH 5.0–6.5, with a higher pH for those samples containing higher amounts of Al in solution (due to pH-increasing effects of the corrosion reactions). After the removal of the specimens by cleaned plastic tweezers and the pH measurement, all solution samples were acidified to a pH of <2 with 65% ultrapure nitric acid (VWR). The cleaned plastic tweezers were also immersed for some seconds into the blank solution samples to account for any contamination. The 65% nitric acid used to acidify the samples was of puriss. p.a. grade (ultrapure). Nitric acid 1%, which was diluted from 65% nitric acid with ultrapure water, was used as diluent, for standard and quality control sample preparation, and for the 0 μg/L standard calibration point. (More analytical details are given in Appendix S1.) All water used was ultrapure water (Millipore, Solna, Sweden, resistivity of 18.2 MΩcm). Ultrapure water is abbreviated MQ. Control duplicate experiments on background concentrations in the different patch-test substances, the metal-free lacquer, and the paper filters without any Finn chambers were also performed.

2.2 Atomic absorption spectroscopy (AAS) and inductively coupled plasma sector field mass spectrometry (ICP-SFMS)

Analysis was conducted with graphite furnace AAS (GF-AAS; AAnalyst 800, Perkin Elmer) with a detection limit (LOD) of <17.4 μg Al/L, further specified for all measurements in Appendix S1. The analysis was performed with triplicate readings and <10% standard deviation (SD) was observed for all samples. The calibration was based on calibration standards in ultrapure 1% nitric acid. Mg(NO₃)₂ acidified with HCl and Ca(NO₃)₂ were used as matrix modifiers. The recovery of quality control samples of 15, 30, 60, and 100 μg Al/L in acidified ASW was acceptable, between 96% and 114%. Regularly analyzed (measured every fifth sample) quality control samples in 1% nitric acid throughout the analysis gave also acceptable (85%–110%) recovery. All samples in artificial sweat had to be diluted up to 100 times to be within the calibration range. Most sample solution concentrations were significantly higher compared with the LOD. Analysis of some of the samples was furthermore performed by ICP-SFMS by ALS Scandinavia, Luleå, Sweden. Samples were diluted 20-fold with 0.14 M HNO₃ (SP grade) in distilled, deionized water. An ICP-SFMS instrument (ELEMENT², ThermoScientific, Bremen, Germany) using a combination of internal standardization (indium added to all solutions at 2 μg/L) and external calibration were used for analysis. The method LOD determined as three times the relative SD for synthetic blanks (n = 5) was 2.2 μg/L. Further analytical details, including corresponding blank concentrations, limits of detection, and exact recovery values, are given in Appendix S1, together with the raw data.

2.3 Calculations and data presentation

The amount of Al release is presented in the unit μg/cm² and calculated by:

\[
\text{Al release (μg/cm}^2\text{)} = \frac{\text{sample conc. (μg/L)} - \text{average of blank conc. (μg/L)}}{A(\text{cm}^2)} \times 0.00157 \text{ L}
\]

where conc. is the concentration, the average of blank concentrations are based on the corresponding blank samples, and A is the exposed chamber surface area (0.157 cm² for most Finn chambers, and 0.785 cm² for the back-side-sealed Finn chambers Aqua and their control Al Finn chambers – batch 2, Table 1).

2.4 Optical microscopy

Ten unexposed Finn chambers and 3 of batch 2 exposed to ASW were inspected visually and by optical microscopy, showing a clear difference with a slightly brownish appearance of the exposed Finn chambers, while all unexposed Finn chambers appeared shiny metallic. This brownish discoloration after exposure to ASW was distributed over the whole exposed surface and there was no specific discoloration along the lacquer, which was used to seal the back side of these Finn chambers, indicative of no crevice corrosion. A Leica DM2770M light optical microscope equipped with a Leica DFC295 camera was used to take representative images at different magnifications up to 100 times (Appendix S1, Table S1).

2.5 Statistical analysis

A Student’s t-test of unpaired data with unequal variance (KaleidaGraph 4.0) was used to calculate whether differences among samples were statistically significant (P-value <0.05).

3 RESULTS

Al release testing from Finn chambers of batch 1 is shown in Figure 1A (left). The Al release into ASW was between 125 and 350 times larger as compared to the release into ultrapure water (P < .05). Although the release from noncleaned and cleaned Finn chambers was not significantly different (P = .8), the release from ground and cleaned Finn chambers was 2-fold larger as compared to non-ground Finn chambers (P < .05) independent of cleaning. The Al release from ground and cleaned Finn chambers of batch 1 corresponded approximately to the patch-test dose of 0.2% AlCl₃·6H₂O in pet. in the IQ Ultra chambers (for exact calculation, see Appendix S1), whereas the release of non-ground Finn chambers corresponded to 0.1% AlCl₃·6H₂O in pet., Figure 1A. Figure 1B shows differences in Al release from non-cleaned (as-received) Al Finn chambers of three different batches and Finn chamber Aqua, a glue- and paper-coated Al chamber. The difference between batch 1 and 3 was statistically significant (P < .001), and the difference between batch 2 and 3 was the greatest (about 30-fold). Batch 1 and 3 released significantly higher Al as compared with the Finn chambers Aqua (P < .01). Between 16- and 4100-fold more Al was released from the Al Finn chambers as compared to the
Finn chambers Aqua. Figure 1B. Clear signs of pitting corrosion were detected on the Finn chambers of batch 2 after exposure to ASW by means of optical microscopy, Appendix S1 (Figure S1).

Figure 2 shows the released amount of Al from empty Finn chambers (batch 3), empty Finn chambers (batch 3) with paper filters, and from the same Finn chambers (batch 3) with 32 different patch-test substances applied. The paper filter resulted in slightly lower, but not statistically significantly lower, release of Al from the Finn chambers. Most patch-test substances resulted in lower \( P < .05 \) release of Al from the Finn chamber as compared with an empty Finn chamber or a Finn chamber with paper filter (Figure 2). Some patch-test substances resulted in a non-significantly higher release of Al as compared to empty Finn chambers: cobalt(II) chloride hexahydrate 1% pet. (2-fold higher release), Myroxylon pereirae resin 25% pet. (13-fold higher release), and palladium chloride 2% pet. (8-fold higher release). Caine mix II 10% pet. and sodium tetrachloropalladate hydrate 3% pet., would correspond to an Al patch-test concentration of 0.5% AlCl\(_3\) in IQ Ultra chambers.

Background concentrations of all patch-test substances, the paper filters, and the metal-free lacquer used to seal the back side of the Finn chambers Aqua and batch 2 were all found to be negligible (<3 μg/L) and close to blank concentrations (Table S3, Appendix S1), as compared to the sample concentrations with Finn chambers of batch 3 (591–21 536 μg/L) (Table S2, Appendix S1).

### 4 DISCUSSION

Al belongs to the passive metals that are protected by a thin surface oxide that hinders corrosion and dissolution effectively in neutral aqueous solutions.\(^ {15} \) However, Al metal is susceptible to localized corrosion and sometimes other types of corrosion in salt solutions, solutions containing certain anions and organic acids, in contact with other metals, and strongly acidic or alkaline solutions.\(^ {16} \) Chlorides have particularly strong effects on localized corrosion of Al metal,\(^ {15, 16} \) which can explain the high release from Finn chambers observed in...
this study in the presence of chloride-containing patch-test substances and in ASW as compared to pure water.

The release of metals from passive metals is further strongly influenced by the surface preparation or storage conditions of the metals prior to testing (for as-received surfaces). Generally, longer storage time and a more humid, warmer, and acidic storage atmosphere will result in lower subsequent release of metals when tested without any further surface preparation (as-received). In this study, three different batches of Finn chambers with slightly different (unknown) age and storage conditions were tested and showed partially statistically significant (up to 30-fold) differences in Al release at similar test conditions for as-received (non-treated) Al Finn chambers. This result is interesting, as it could possibly explain the difference observed in reactivity to empty Finn chambers observed in different studies. Further studies are required to understand this baseline variation of Al release from empty Finn chambers. In addition to different transport and storage conditions, the Al release for different batches could also be influenced by factors caused and controlled during manufacturing, such as inclusions, differences in impurities, or in internal stresses (residual tensile or compressive stresses originating from external stresses during manufacturing such as bending). Another difference is the sealing of the back side. The batch that served as reference for the Finn Aqua chambers (batch 2), and was therefore sealed on the back side, released the highest amounts of Al both in absolute concentration and dose per surface area (Appendix S1). It cannot be determined in this study whether this was caused by a difference in storage conditions, batch, or an effect of the sealing procedure. Sealing a passive metal susceptible to localized chloride-induced corrosion poses a risk of causing a micrometer-thick crevice that might serve as initiation site for localized corrosion and therefore accelerate the process of Al release, but no crevice corrosion was observed visually and by optical microscopy in this study. Instead, the corrosion type resembled pitting corrosion and was distributed over the entire exposed surface (Appendix S1, Figure S1).

Several of the investigated patch-test substances contain chlorine, as covalent bound chloride in organic molecules, as easily dissociated chloride in metal salts, or as hydrochloric acid (Appendix S1). The amount of applied chloride varied from 0.00071 mg (substance 22, methylchloroisothiazolinone/methylisothiazolinone 0.02% aq.) to 0.256 mg (substance 8, quinoline mix 6% pet.), as compared to the chloride content of 7.85 mg in the applied volume of ASW (Appendix S1). The amount of chloride is hence negligible as compared to the amount of chlorides that can be expected to originate from sweat under patch-test conditions. However, its physical presence on the surface and its chemical speciation vary. For instance, the chlorides of metal salts can be expected to be more labile (available for reactions), since they are supposed to dissociate, as compared to the chlorides included in organic molecules. In addition, hydrochloric acid, as weakly bound to two of the three compounds in Caine Mix II (Appendix S1), is particularly corrosive to Al. In addition, several of the patch-test substances contain high amounts of acids, for example, M. pereirae resin 25% pet. The exact corrosion mechanism of the different patch-test substances remains to be investigated.

In this study, several of the patch-test substances induced strong, but not statistically significantly, increases of Al release, which in all cases was because the three specimens released varying amounts of Al. Only one of three specimens with cobalt(III) chloride hexahydrate 1% pet. was increased (0.66, 0.59, and 4.7 μg/cm²), whereas two of three specimens showed increased Al release for palladium chloride 2% pet. (0.92, 13, and 13 μg/cm²) and for M. pereirae resin 25% pet. (23, 17, and 2.4 μg/cm²). This can be interpreted as conditions induced experimentally being close to metastable corrosion conditions, shifting between passive and active conditions (pitting corrosion) by very slight differences in local chloride concentrations or surface conditions. In contrast, the three specimens with Caine Mix II 10% pet. (2.5, 2.5, and 2.8 μg/cm²) and with sodium tetrachloropalladate hydrate 3% pet. (27, 19, and 19 μg/cm²) varied less and hence showed a statistically significantly increase compared with the five Al Finn chambers without any patch-test substance (1.6, 1.0, 0.96, 0.99, and 1.0 μg/cm²).

This study can be considered a first screening study that can serve as a base for further detailed studies that are required to understand whether and when Al release from Al Finn chambers or its corrosion reactions are clinically relevant, either for Al-allergic individuals or due to chemical interactions with patch-test substances. It is clear from this study, which has been well-controlled with several background control measurements, that the Al release is a consequence of pitting corrosion of the Al surface induced by the patch-test substances and species in ASW. This study is limited as an in vitro study, and because the manufacturing, storage, and transport conditions of the chambers could not be controlled. The back side of the chambers has been included in this study of the different patch-test substances, which is not relevant for patch testing. On the other hand, sealing the back side might pose a risk of crevice corrosion under the experimental conditions of this study. However, crevice corrosion was not observed in this study.

Considering the fact that neither AlCl₃·6H₂O in pet. (2% or 10%) nor any other Al patch-test substance is currently included in the Swedish national or in international baseline series, it might be difficult to recognize Al allergy, and hence there is a risk of false-positive reactions, and consequent misdiagnosis, to other haptens in Al-sensitized individuals. Al allergy is relatively common in some countries and age groups (about 1% of general population) and might therefore pose a serious risk of jeopardizing a correct diagnosis using patch testing with Finn chambers. Several Swedish studies are currently ongoing to investigate whether Al release from Finn chambers could influence diagnostic outcomes. This has also been discussed recently for isolated palladium allergy. From a chemical point of view, release of Al from Al Finn chambers could be of concern for current patch-test diagnostic outcomes.

5 | CONCLUSION

The amount of Al released from empty Al Finn chambers corresponded to a skin dose of approximately 0.03%–0.5% Al chloride hexahydrate applied in plastic chambers. Finn chambers Aqua
released significantly lower (16- to 4100-fold) amounts of Al. Although most patch-test substances reduced the release of Al from the Al Finn chambers, some substances significantly increased the release of Al from the Finn chambers, most notable for Caine mix II 10% pet., M. pereirae resin 25% pet., and sodium tetrachloropalladate hydrate 3.0% pet. (corresponding to 0.5% Al chloride hexahydrate).

The release of Al from Finn chambers should be considered in further development of diagnostic patch testing. We strongly recommend patch testing of Al chloride hexahydrate 10% pet. in a plastic chamber as a control substance if Al Finn chambers are used for patch testing.

CONFLICTS OF INTERESTS

The authors declare they have no conflicts of interest.

AUTHOR CONTRIBUTIONS

Yolanda Hedberg: Conceptualization; formal analysis; funding acquisition; investigation; methodology; resources; supervision; validation; visualization; writing-original draft; writing-review and editing. Zheng Wei: Conceptualization; formal analysis; investigation; methodology; writing-original draft; writing-review and editing. Mihály Matura: Conceptualization; funding acquisition; resources; supervision; writing-original draft; writing-review and editing.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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