Nanoparticles reduce nickel allergy by capturing metal ions

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Approximately 10% of the population in the USA1,2 suffer from nickel allergy3–5, and many are unable to wear jewellery or handle coins and other objects that contain nickel6–10. Many agents have been developed to reduce the penetration of nickel through skin11,12, but few formulations are safe and effective13–15. Here, we show that applying a thin layer of glycerine emollient containing nanoparticles of either calcium carbonate or calcium phosphate on an isolated piece of pig skin (in vitro) prevents the penetration of nickel ions into the skin. The nanoparticles capture nickel ions by cation exchange, and remain on the surface of the skin, allowing them to be removed by simple washing with water. Approximately 11-fold fewer nanoparticles by mass are required to achieve the same efficacy as the chelating agent ethylenediamine tetraacetic acid. Using nanoparticles with diameters smaller than 500 nm in topical creams may be an effective way to limit the exposure to metal ions that can cause skin irritation.

Metal-induced contact dermatitis is a common allergic reaction to minute amounts of metal ions or particles in direct contact with the skin. An ideal preventative treatment for nickel-induced contact dermatitis is an emollient that can be applied topically to effectively capture nickel ions over a wide range of pH values (for example, in the presence of sweat), without absorbing through the skin or causing any toxic side effects. Because of their large surface-area-to-volume ratio, nanoparticles have been used as chelating agents for a variety of applications including sensors16,17.

In general, soft acids and bases have a lower charge state and larger radius than hard acids and bases. Nickel is a soft acid18 and soft acids and bases principle proposed by Pearson20, we studied the nickel-chelating ability of several soft bases including phosphate and carbonate ions. Previously, Kuo and colleagues have demonstrated that without permeabilization enhancers such as oleic acid and calcium carbonate (CaCO3) or calcium phosphate (CaPO4) nanoparticles (from the list of ‘generally recognized as safe’ (GRAS) agents of the United States Food and Drug Administration) to minimize skin penetration, showing that when applied with an emollient to the skin of pigs (in vitro) and mice (in vivo), these nanoparticles can efficiently bind and reduce skin exposure to nickel ions.

To evaluate the efficiency with which nanoparticles bind to nickel ions in solution, CaCO3 or CaPO4 nanoparticles were suspended in a NiSO4 aqueous solution (0.2 M, 5.25%, wt/vol) made with artificial sweat (containing minerals, metabolites and 20 amino acids; pH 6.1). The use of artificial sweat is an industry standard for testing the release of metal ions such as nickel from jewellery22. After 48 h incubation, the nickel concentration in the supernatant was measured using an inductively coupled plasma atomic emission spectrometer (ICP-AES). The data summarized in Fig. 1a show that CaCO3 and CaPO4 nanoparticles efficiently capture nickel and significantly reduce nickel concentration (>99%). Energy-dispersive X-ray (EDX) analysis of these particles shows the characteristic peaks (Supplementary Fig. S1) corresponding to calcium and nickel at 3.6 (Ca-Ka), 0.85 (Ni-La) and 7.47 keV (Ni-Ka), confirming that the nanoparticles have the ability to sequester nickel. In a separate experiment, nickel wires (acting as a source of nickel ions) were immersed in artificial sweat containing a suspension of either CaCO3 or CaPO4 nanoparticles. After 72 h, ICP-AES analysis of the suspension showed that samples containing nanoparticles had ~99% fewer nickel ions than those without particles, suggesting that the nickel ions released from the wires were effectively captured by the particles (Fig. 1a). The nanoparticles could also efficiently capture other metal ions such as palladium, cadmium and cobalt, which may also provoke allergies (Fig. 1a).

It has been shown that nanoparticles larger than 20 nm do not penetrate skin in the absence of permeabilization enhancers21, so we chose to use nanoparticles that were about three times this size to prevent any possible skin penetration. Importantly, CaCO3 particles smaller than ~500 nm in diameter were more efficient than particles larger than ~500 nm at chelating nickel ions in NiSO4 salt-containing artificial sweat (Table 1; for size distribution see Supplementary Fig. S2), and also at chelating nickel ions directly from a nickel wire that was coated with CaCO3 particles and placed in artificial sweat (Fig. 1b; see Supplementary Fig. S3 for coating methodology). This was probably owing to the high surface area of nanoparticles with diameters less than ~500 nm. Similar to the inverse relationship between CaCO3 particle size and nickel chelation, we found that ~100-nm-sized CaPO4 nanoparticles coated on nickel wires could also efficiently capture nickel ions released from the wires (see Supplementary Fig. S4 for scanning electron microscopy (SEM) images and particle size distribution). Specifically, concentrations of nickel released from the uncoated and CaPO4-coated nickel wires after 48 h incubation were 812 and 16 ppm, respectively.

The nickel binding energies (BE) were 856.1, 854.8 and 854.2 eV for NiSO4, nickel-captured CaPO4 and nickel-captured CaCO3 particles, respectively (Fig. 1c, analysed by X-ray photoelectron spectroscopy, XPS). The BE of nickel-captured CaCO3 particles (854.2 eV) matched the reported BE of NiCO3 (ref. 23), suggesting the formation of NiCO3 as nickel was sequestered by the CaCO3 particles. Similarly, the BE of nickel-captured CaPO4 particles matched with that reported for NiPO4, suggesting the chelation of nickel with phosphates24. The potential existence of physically adsorbed nickel is possible; however, it probably represents only a
small percentage of bound nickel given the presence of small shoulder peaks in the XPS spectra (Fig. 1c).

A significant reduction in chelation efficiency (∼40% less) was observed for the hard-acid cobalt ions (Fig. 1a) when compared to the soft-acid nickel. Despite the reduced surface area of the CaCO₃ observed for the hard-acid cobalt ions (Fig. 1a) when compared to experiments show that 0.5 g of CaPO₄ particles can scavenge the wires coated with different sized CaCO₃ nanoparticles. Dermatitis in humans through the formation of a stoichiometric positive control because it is able to abrogate nickel-induced contact suggesting that cation exchange does occur during chelation.

Calcium ion is released (Fig. 1d) in the presence of nickel or zinc, would trigger the release of calcium. Indeed, a 10-fold excess of calcium ion is released (Fig. 1d) in the presence of nickel or zinc, suggesting that cation exchange does occur during chelation.

Ethylenediamine tetraacetic acid (EDTA) is commonly used as a direct chelation of nickel with either CaPO₄ or CaCO₃ particles not typically promote counterion release from the salt, we postulated the capture of cobalt ions, as was anticipated by the ‘hard and soft acids and bases’ principle. Thus, the nature of the metal and the capturing agent (soft or hard acid/base) affects the efficiency of the sequestering process. Given that adsorption of ions onto a salt does not typically promote counterion release from the salt, we postulated that direct chelation of nickel with either CaPO₄ or CaCO₃ particles would trigger the release of calcium. Indeed, a 10-fold excess of calcium ion is released (Fig. 1d) in the presence of nickel or zinc, suggesting that cation exchange does occur during chelation.

The upper surface of skin (stratum corneum) is composed of numerous layers of dead cells (thickness, ∼10–40 μm) without a blood supply. Thus, confining nickel to the surface through complexation with nanoparticles allows one to easily rinse away the complex from the skin and prevent contact-induced dermatitis. To evaluate the ability of CaCO₃ (∼70 nm) or CaPO₄ (∼100 nm) nanoparticles to prevent penetration of nickel ions into the skin, nanoparticles were dispersed (20% wt/wt) in glycerine (an emollient), and 50 μl of this suspension was applied as a thin layer on top of isolated pig skin. Subsequently, 50 μl of a high concentration (0.2 M, 5.25% wt/vol)) of NiSO₄ solution was added on top of the skin and incubated for 5 h (Fig. 2a). In control experiments, 50 μl of glycerine (without nanoparticles) was applied to the pig skin, and in all groups (experimental and control) 50 μl of NiSO₄ (0.2 M, 5.25% wt/vol) solution was applied. The solution was not permitted to contact

### Table 1 | Effect of nanoparticle size on efficiency to bind nickel.

<table>
<thead>
<tr>
<th>Size of CaCO₃ particles (μm)</th>
<th>Surface area (m² g⁻¹)</th>
<th>Concentration of nickel (ppm)</th>
<th>% of Ni decrease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without particles</td>
<td>-</td>
<td>25,000</td>
<td>0.0</td>
</tr>
<tr>
<td>0.07</td>
<td>28,686</td>
<td>2,475</td>
<td>90.1</td>
</tr>
<tr>
<td>0.5</td>
<td>20,674</td>
<td>5,412</td>
<td>78.3</td>
</tr>
<tr>
<td>1</td>
<td>10,900</td>
<td>7,675</td>
<td>69.3</td>
</tr>
<tr>
<td>3</td>
<td>7,732</td>
<td>8,418</td>
<td>66.2</td>
</tr>
</tbody>
</table>

Equal amounts (0.5 g) of CaCO₃ particles (∼70, ∼500, ∼1,100 and ∼3,000 nm) were combined with 11.2% (wt/vol) NiSO₄ (25,000 ppm of nickel in artificial sweat) after 24 h, particles were removed and the concentration of nickel in the supernatant was measured using ICP-AES. In all cases, values are the average of three independent experiments and all standard deviations are <5% of the average values.
the underside of the skin. The skin samples were then vertically sectioned and examined for elemental mapping (SEM-EDX) to visualize the location of the nickel, or washed with deionized water, then vertically sectioned and subjected to SEM-EDX (Fig. 2b) to visualize the location and amount of nickel remaining in the skin (that is, in analogy to assessing the impact of handwashing or showering). For these experiments, non-coated skin samples and samples coated with vehicle only (glycerine) were used as controls.

The presence of a coating containing CaCO$_3$ or CaPO$_4$ nanoparticles significantly reduced skin exposure to nickel ions. In skin samples coated with nanoparticles, no detectable nickel was found to have penetrated through the skin (see Fig. 2b, ‘before washing’). Furthermore, the particles were retained on the surface of the skin (Fig. 2b) and could be removed easily with water without leaving any residue containing nickel or nanoparticles. In contrast, elemental mapping images of skin from uncoated samples and those coated with glycerine only revealed that nickel had permeated through the skin. Absorption of nickel in the skin (with and without nanoparticle coating; for SEM images see Fig. 3a) was quantified using vertical Franz diffusion cell experiments (Supplementary Fig. S5; see Methods). Following 48 h incubation in 0.05 M NiSO$_4$, the bare skin (untreated, full-thickness pig skin) and glycerine-only-coated skin absorbed ~200-fold more nickel compared to skin coated with either CaCO$_3$ or CaPO$_4$ nanoparticles (Fig. 3b).

With a view to clinical application, it is important to show that the reduction of nickel exposure in vitro through GRAS-based nanoparticles can moderate the inflammatory response. All animal work was performed in collaboration with Biomodels LLC. Nickel-sensitized C3H/HeJ female mice that exhibited mild erythema were exposed to nickel in the presence and absence of a CaPO$_4$-particle coating. Cutaneous toxicity was scored on a scale from 0 to 5 as shown in Fig. 4a at 0, 24, 48 and 72 h following nickel exposure. Although glycerine (the vehicle for applying nanoparticles) is commonly used as a skin moisturizer and for treating skin irritation/inflammation, it acts as a permeation enhancer for hydrophilic agents, especially in damaged skin.

Thus, we examined if glycerine would enhance nickel absorption into nickel-sensitized skin with mild erythema. Figure 4b shows that glycerine promoted elevated dermatitis scores when compared to the scores for untreated control mice, but the application of CaPO$_4$ nanoparticles in glycerine before nickel application minimized the dermatitis response (Fig. 4c). These results suggest that glycerine exacerbates mild skin inflammation by promoting increased nickel absorption, whereas the presence of CaPO$_4$ particles significantly inhibits nickel exposure; this is also supported by in vitro studies.

Given that glycerine alone elevated the dermatitis scores in this study, it is more appropriate to compare the nanoparticle group (which contains glycerine) with the glycerine vehicle group than with the untreated group. In the application of nanoparticles to broken skin, one could consider using an alternative delivery vehicle that does not act as a permeation enhancer like glycerine. Overall, in vitro and in vivo results show that GRAS-based nanoparticles can significantly reduce exposure to metal ions such as nickel when applied as a coating on the skin within a simple emollient. For practical applications, the nanoparticle approach would involve daily application and removal of the coating. Thus, the barrier
function from the particles would need to last 12–24 h (depending on the frequency of application).

In conclusion, we have shown that GRAS-based CaCO₃ or CaPO₄ nanoparticles that contain soft base ions can efficiently capture soft acid metal ions such as nickel. The size of the nanoparticles plays a pertinent role in efficacy; particles less than 500 nm in size efficiently bind nickel owing to their large surface area, and carbonates/phosphates capture nickel more efficiently than cobalt owing to their higher affinity towards soft acid nickel than hard acid cobalt. Both in vitro and in vivo results suggest that nanoparticles can effectively prevent the penetration of nickel into the skin, and may therefore abrogate nickel-induced contact dermatitis. Thus, the use of GRAS-based nanoparticles within topical compositions may represent an effective approach to limit skin exposure to metal ions, which should be beneficial both occupationally and socially to the tens of millions of people who suffer from metal-induced contact dermatitis.

Methods
See Supplementary Materials for a detailed description of materials, skin preparation procedure, SEM, EDX and ICP-AES experimental procedures.

**Figure 3** | In vitro experiment using isolated pig skin. a, SEM image of untreated (top) and CaCO₃ nanoparticle-coated (bottom) pig skin showing the presence of the nanoparticle coating on the skin. b, Graph showing the efficacy of nickel capture by nanoparticles. CaCO₃ or CaPO₄ particles in glycerine were applied to pig skin, placed into a diffusion chamber and subsequently exposed to nickel ions (0.05 M, 1.3% (wt/vol) NiSO₄). After 48 h, the skin was removed, and unbound particles and nickel were removed by washing with phosphate buffer saline. Subsequently, skin was dissolved in a 1:1 mixture of HNO₃ and H₂SO₄ and subjected to H₂O₂, and then nickel concentration in the solution was quantified using ICP-AES. In all cases, values are the average of three independent experiments and all standard deviations are <5% of the average values.

**Figure 4** | In vivo nickel challenge experiments. All animals were sensitized with NiSO₄ solution. On day 14, mice were challenged with 0.4% NiSO₄·6H₂O into the left rear footpad, and saline was injected into the right rear footpad as a control. Swelling was measured with digital calipers up to 72 h post nickel challenge. Animals that exhibited sensitivity to nickel were randomized into three groups and on day 21, 45 μl of 20% NiSO₄·6H₂O solution was applied after applying either nothing ‘untreated’, ‘glycerine’ or ‘NPs-in-glycerine’ coating. Mice in the ‘untreated’ group received nickel without addition of glycerine or nanoparticles. Mice in the ‘glycerine (vehicle)’ group were treated daily with glycerine only before the application of nickel ions. Mice in the ‘CaPO₄ NPs in glycerine’ group were treated with glycerine containing ≏100 nm CaPO₄ nanoparticles (20% wt/wt) daily before the application of nickel ions. All animals were evaluated at 0, 24, 48 and 72 h for nickel sensitivity (dermatitis score). a, Dermatitis was evaluated by blinded observers on a scale from 0 to 5. b, Temporal dermatitis score comparisons of glycerine (vehicle) treated and untreated mice (*P < 0.05). c, and between mice treated with CaPO₄ nanoparticles in a glycerine coating and mice treated with glycerine only (vehicle) (c) show that the nanoparticle coating reduces the inflammatory response (*P < 0.05).
Preparation of CaCO₃ or CaPO₄ particles coated nickel wire. CaCO₃ or CaPO₄ particles (0.5 g) were suspended in double-distilled water, vortexed for 15 min at room temperature, and nickel wires (length, 2 cm; diameter, 0.5 mm; weight, 40 mg) were then immersed for 10 min. Subsequently, wires were removed and washed twice with double-distilled water and air-dried.

Quantification of nickel release from nickel wires. Nanoparticle-coated nickel wires were incubated in 2 ml artificial sweat, at regular time points (1, 2, 3 and 4 days) A volume of 100 μl of this solution was diluted 1,000 times with 2% vol/vol HNO₃ (Sigma Aldrich) aqueous solution, and subjected to ICP-AES (Horiba Jobin Yvon, Activa S) to measure the nickel concentration.

Quantification of metal sequestered by the nanoparticles. Two sets of experiments were performed to quantify the amount of nickel (either from NiSO₄ solution or released from nickel wire) sequestered by the CaCO₃ or CaPO₄ nanoparticles. Set 1. Nanoparticles (0.5 g) were suspended in 0.2 M NiSO₄ aqueous solution; after incubation for 4 h, particles were centrifuged (20,000 r.p.m. for 15 min) and the supernatant was collected and subjected to ICP-AES (Horiba Jobin Yvon, Activa S) to measure the nickel concentration.

Surface area measurements. Specific surface area analysis was performed using the Brunauer-Emmett-Teller (BET) method of nitrogen gas adsorption/desorption.

In vivo nickel sensitization and nickel challenge experiments. Before sensitization, C3H/HeJ female mice were shaved to remove abdominal hair. All animals were sensitized (day 0) by the abdominal application of 6 mm pads containing two layers of 0.3 mm filter backing paper (Biorad) soaked in 45 μl of an aqueous 20% solution of NiSO₄·6H₂O. These pads were covered with numerous layers of Opsite, and remained in place for 7 days. On day 14, mice were challenged by injecting 10 μl of 0.4% NiSO₄·6H₂O into the right foot using a Hamilton syringe and 30G needles. Saline (10 μl) was injected into the right foot as a control. Swelling was measured with digital calipers at 0, 24, 48 and 72 h for all animals. Animals that exhibited sensitivity to nickel were randomized into three groups (eight mice per group). On day 21, following application of the nanoparticle coating, all animals had a 6-mm pad soaked in 45 μl of an aqueous 20% solution of NiSO₄·6H₂O applied to the abdomen, and protected with Opsite. These pads were removed and replaced daily to allow for the evaluation of nickel sensitivity at 0, 24, 48 and 72 h post initial application. Animals in Group 1 were untreated (without addition of glycerine or nanoparticles), animals in Group 2 (only glycerine vehicle) were treated with glycerine daily before application of nickel, and animals in group 3 (nanoparticles with glycerine vehicle) were treated with glycerine containing CaPO₄ nanoparticles (20% wt/wt) daily before application of nickel. All animals were evaluated at 0, 24, 48 and 72 h for nickel sensitivity (dermarisc score). The study was stopped five days after the nickel challenge was initiated as animals began to lose weight. With respect to total body weight at the start of the study, untreated mice lost 7.7 ± 9.3%, mice treated with glycerine (vehicle only) lost 14.8 ± 10.5%, and mice treated with CaPO₄ nanoparticles and glycerine lost 0.3 ± 2%.

Statistical analysis. All data were compared using Tukey’s HSD (ANOVA with post hoc analysis) test at the significance level of 95% (P < 0.05).

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References

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Author contributions
P.K.V. and J.M.K. conceived and designed the experiments, analysed the data and co-wrote the manuscript. R.R.A. helped design the experiments and write the manuscript.

Additional information
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