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Chitosan−Glycerol Gel as Barrier Formulation for Metal Allergy

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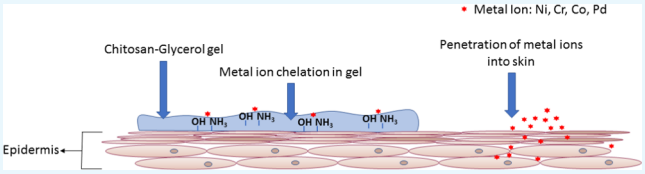
ABSTRACT: Metal-induced allergic contact dermatitis, particularly nickel, affects over 10% of the general population. Herein, chitosan−glycerol gel as protective barrier formulation was synthesized by neutralization reaction with an aim to reduce metal-ion diffusion into the skin to prevent allergy. Active functional groups in chitosan−glycerol gel were able to capture allergenic metal ions present in artificial sweat solution. The efficacy of the barrier formulation against nickel-ion penetration was evaluated ex vivo using pig skin. We found that the percutaneous absorption of nickel ion reduced by ∼98% when chitosan−glycerol gel was used as a barrier formulation.



1. INTRODUCTION

Metal contact dermatitis (MCD) or metal allergy is an inflammatory disease that is caused by exposure to metal ions of nickel, cobalt, chromium, palladium, etc.[1] Common sources of allergenic metals are belt buckles, buttons, doorknobs, jewelry, laptops, mobile phones, wrist watches, etc.[2−4] Being electrophilic, metal ions can ionize and bind to carrier proteins causing allergic responses typical of rashes, swelling, and pain.[5] Topical barrier formulations are common prophylaxis measures for MCD that function as a protective layer against metal allergens. These barrier formulations contain active ingredients that scavenge the metal ions to form stable metal complexes. The existing barrier formulations contain molecular chelators such as ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepenta-acetic acid (DTPA), and clioquinol, or nanoparticle-based formulations of $CaCO_3$ and $CaPO_4$.[6−8] However, molecular chelator-based formulations of EDTA and clioquinol are allergens in themselves, hence not widely preferred.[9−11] Praveen et al. demonstrated $CaCO_3$ and $CaPO_4$ nanoparticles affinity for allergenic metal ions and was effective in preventing penetration of nickel ion.[8] However, homogeneous dispersion of nanoparticles could be a challenge since nanoparticles tend to agglomerate due to high surface area and absence of surface modification or stabilizers.

Chitosan is a polycationic natural polysaccharide derived by partial deacetylation of chitin.[12] Chitosan has wide pharmaceutical applications due to its characteristic properties such as biocompatibility, nontoxicity, and antimicrobial and tissue regeneration properties.[13] The reactive amino and hydroxyl groups in chitosan aid in chelation of various heavy-metal ions, and are thus used as adsorbent of toxic heavy-metal ions.[14] An ideal barrier formulation in addition to its function as a protective skin barrier should also help in hydration of skin to treat symptomatic effects of MCD such as drying, scaling, and skin fissures.[15] Chitosan−glycerol (Cs) gel as a barrier formulation was explored, where chitosan functions as a scavenger of metal ions and glycerol as humectant and plasticizer.[16]

2. RESULTS AND DISCUSSION

Alloys of nickel, cobalt, chromium, and palladium are important material with extensive engineering applications. The ubiquity of these alloys in our daily lives makes it practically impossible to avoid its exposure. With increasing number of metal allergy cases reported, it is imperative to find an efficient, cost-effective, and safe prophylactic for metal allergy. In this study, we have evaluated the scope of chitosan−glycerol gel as an active barrier formulation against major allergenic toxic metals ions that causes metal contact dermatitis.

The efficacy of the Cs gel was determined by analysis of chelated metal ions in chitosan matrix using energy-dispersive spectroscopy (EDS) (Figure 1). The presence of nickel in Cs gel was confirmed with characteristic peaks of Lα at 0.851 keV, Kα at 7.480 keV, and Kβ at 8.267 keV. Chromium was confirmed with characteristic peaks of Cr at Kα at 5.415 keV, Kβ at 5.947 keV, and Lα at 0.573 keV. Similarly, cobalt characteristic peaks were displayed at Lα at 0.776 keV and palladium at Lα at 2.838 keV.

The amount of chelated metal to chitosan−glycerol gel was quantified by inductively coupled plasma-mass spectroscopy (ICP-MS) by calculating the difference between the initial and final concentrations of metal ions present in the supernatant after 14 h incubation of Cs gel in the artificial sweat solution containing metal ions. The bar chart in Figure 2 shows that Cs gel was effective in absorbing >99% of the metal ions from the solution in the case of nickel, cobalt, and palladium and lower in the case of chromium. The lower chelation efficiency in the case of chromium can be explained by the dual competition between CrO_4^{2-} and OH^- to the surface of chitosan, where OH^- is predominant.[17,18] These results were superior to those of DTPA-based formulations that had not been effective against chromium and palladium.[19]

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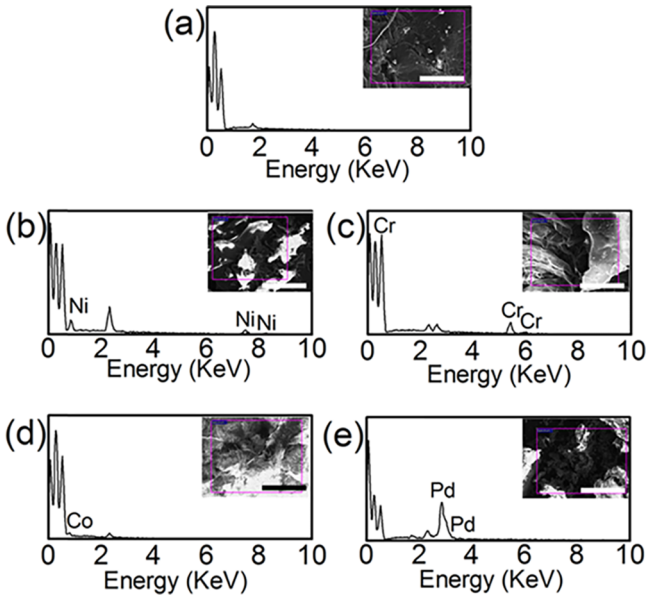

Figure 1. Energy-dispersive spectroscopy of (a) Cs, (b) Cs−Ni, (c) Cs−Cr, (d) Cs−Co, and (e) Cs−Pd (inset: scanning electron microscopy (SEM) images (scale bar 300 μm)).

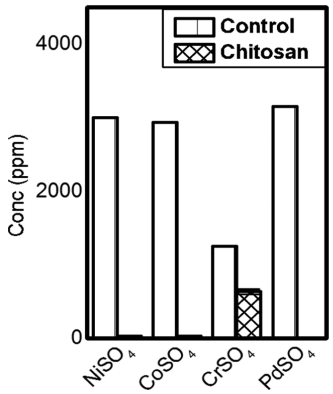

Figure 2. Metal chelation efficiency of chitosan−glycerol gel.

The structural analysis of Cs by X-ray diffraction (XRD) in Figure 3a displayed chitosan's characteristic narrow peak at 20°; however, the peak broadened with minor increase in the case of chitosan−metal complexes, suggesting disordered crystal structure after metal complexation.[20,21] Figure 3b shows the Fourier transform infrared (FTIR) spectra of chitosan and chitosan−metal complexes of nickel, chromium, cobalt, and palladium. Chitosan characteristic peaks are recorded at 3284 cm^{-1} corresponding to −NH and −OH stretching vibrations, 2880−2934 cm^{-1} corresponding to C−H symmetric and asymmetric stretchings, and 1031 corresponding to C−O stretching vibration. After forming metal complexes, the peaks corresponding to −NH and −OH stretching vibrations shift to a lower wavenumber, i.e., 3267, 3252, 3272, and 3253 cm^{-1} for Cs−Ni, Cs−Cr, Cs−Co, and Cs−Pd, resepectively. The shift of peaks corresponding to −NH and −OH stretching vibration is related to coordination bonding between the bonding electron pair on the nitrogen atom in the $-NH_2$ group and the electron in vacant D orbital of transition metal. Metal-complexed chitosan shows broader peaks in the range 900−1200 cm^{-1}, which are also evidence of disordered structure with lower crystallinity.[22,23] The material characterization studies confirm the formation of stable metal−amine or metal−hydroxyl complexes in Cs gel. However, the amine group in chitosan has a pK_a of 6.3; hence, Cs remains soluble at weakly acidic solutions.[24] In the present case, the metal chelation testing was done in the artificial sweat solution (EN 1811:2011) at pH 6.5 considering that the applied gel will encounter only sweat on practical application.

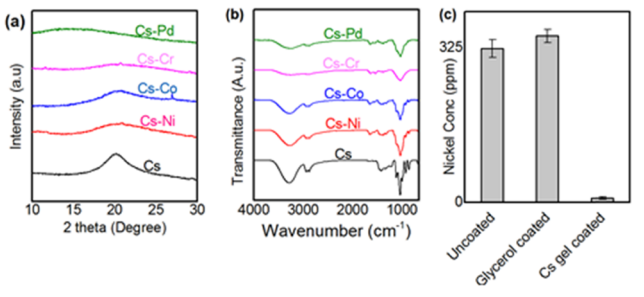

Figure 3. (a) XRD pattern and (b) FTIR spectra of dried hydrogels and metal-complexed gel and (c) ex vivo quantification of percutaneous absorption of nickel ion on pig skin.

Several authors have reported the ability of various metal ions to penetrate through the skin by in vitro model of franz diffusion cell.[25,26] The efficacy of the prepared barrier formulation to prevent metal ions from diffusion through the skin was evaluated by recording the metal ion absorbed in skin in the presence and absence of barrier formulation. Results in Figure 3c display that a significant amount of nickel ions was absorbed onto the pig skin in both uncoated and glycerol-coated samples. The minor increase in percutaneous absorption of nickel ion in glycerol-coated samples relative to uncoated sample can be attributed to glycerol acting as permeation enhancer.[27] The Cs gel-coated sample effectiveness of chitosan−glycerol gel as barrier formulation was confirmed with the significant reduction (~98%) in percutaneous absorption of nickel ions. Also, similar results are expected on metal ions of Co, Cr, and Pd that were proven to have comparable affinity to chitosan−glycerol gel.

3. CONCLUSIONS

In summary, the synthesized active topical barrier formulation of chitosan−glycerol gel exhibits high affinity to allergenic nickel, chromium, cobalt, and palladium ions. Ex vivo permeation study demonstrates that chitosan−glycerol gel was effective in significantly reducing the penetration of nickel ions into the skin, thus potentially reducing the chances of metal allergy.

4. MATERIALS AND METHODS

4.1. Materials. Chitosan of medium molecular weight (190 000−310 000 Da) with 75−85% deacetylation was obtained from Sigma-Aldrich. Acetic acid, ammonium hydroxide, lactic acid, sodium chloride, sodium hydroxide pellets, urea of ACS grade, and glycerol of SQ grade were obtained from Fisher Scientific. Chromium(III) sulfate hydrate, cobalt(II) sulfate heptahydrate, nickel sulfate hexahydrate, and palladium(II) sulfate dihydrate of reagent grade were obtained from Alfa Aesar. All solutions were prepared in Milli-Q water of resistivity not less than 18.2 MΩ cm^{-1}.

4.2. Preparation of Chitosan−Glycerol Gel. One part of acetic acid was mixed with three parts of glycerol to form 1%

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chitosan solution. The solution was neutralized (pH 7) with NaOH to obtain chitosan−glycerol gel. The obtained gel was further washed by centrifugation for 5 min to remove neutralized salts and free solvents. The amino group in chitosan has a pK_a of 6.3; hence, the Cs remains soluble in weakly acidic solutions.

4.3. Metal Chelation Studies. $NiSO_4$ (10 mL, 0.05 M), $CrSO_4$ (7 mL, 0.05 M), $CoSO_4$ (10 mL, 0.1 M), and $PdSO_4$ (9.5 mL, 0.03 M) were made in EN 1811:2011 artificial sweat solution (0.5 wt % NaCl, 0.1 wt % lactic acid, 0.1 wt % urea, pH 6.5 adjusted with NH_3). The chitosan−glycerol gel (4 g) was added to each prepared metal solution and kept under agitation for 14 h. Post incubation, the samples were centrifuged and supernatants were analyzed by inductively coupled plasma-mass spectroscopy (ICP-MS, Thermo Scientific, XSeries 2). Chitosan−glycerol gel (Cs) and metal-complexed gels of nickel (Cs−Ni), cobalt (Cs−Co), chromium (Cs−Cr), and palladium (Cs−Pd) were washed thoroughly with Milli-Q water, dried at 70 °C for 24 h, and powdered in a mortar and pestle. Powdered materials were further characterized by energy-dispersive spectroscopy and field emission scanning electron microscopy (EDS-FESEM, Gemini, Ultra55), X-ray diffraction (XRD, Bruker D8 Advance), and Fourier transform infrared spectroscopy (FTIR, PerkinElmer) to confirm chelation of metal ions to Cs matrix. A 10 nm gold film was sputtered onto to the sample and examined by EDS-FESEM at an electron accelerating potential of 10 KeV. X-ray diffraction patterns were recorded from 10 to 30° with a scanning speed of 1.2° min^{-1} using Cu Kα radiation. The FTIR spectra were recorded in UATR mode in the range of 400−650 cm^{-1}.

4.4. Ex Vivo Permeation Studies. Percutaneous absorption of nickel ions on pig skin was evaluated using Franz static diffusion cell. The pig skin was obtained from local abattoir and treated within 1 h after sacrifice. Subcutaneous fat was removed by surgical blades, and the skin was cut into 2 × 2 cm^2 sections. Franz static diffusion cell (15 mm diameter, Anton Scientific) was washed with aqua regia and Milli-Q water before performing diffusion experiments. The donor chamber of Franz diffusion cell was filled with 2 mL of 0.05 M $NiSO_4$ salt solution, and the receiver chamber with Milli-Q water throughout the experiments. To test the efficacy of chitosan−glycerol gel as barrier formulation, 1 g of the chitosan−glycerol gel was spread on the skin just before the diffusion experiment was performed. All diffusion cell experiments were done for 48 h, after which the skin was removed and washed thoroughly with Milli-Q water to remove unbound nickel ion and/or residual gel. Skin membranes were later digested using 10 mL of concentrated nitric acid and 2 mL of 30% hydrogen peroxide under agitation for 12 h. The solution was heated at boiling point to vaporize all of the liquids. The solid residue obtained was redissolved in 2% nitric acid and analyzed by ICP-MS. Untreated pig skin and pig tissues with 500 μL of glycerol served as controls throughout the diffusion experiments.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Garner, L. A. Contact Dermatitis to Metals. *Dermatol. Ther.* **2004**, 231−327.

(2) Basketter, D. A.; Briatico-Vangosa, G.; Kaestner, W.; Lally, C.; Bontinck, W. J. Nickel, Cobalt and Chromium in Consumer Products: A Role in Allergic Contact Dermatitis? *Contact Dermatitis* **2006**, *28*, 15−25.

(3) Aquino, M.; Mucci, T.; Chong, M.; Lorton, M. D.; Fonacier, L. Mobile Phones: Potential Sources of Nickel and Cobalt Exposure for Metal Allergic Patients. *Pediatr. Allergy. Immunol. Pulmonol.* **2013**, *26*, 181−186.

(4) Jensen, P.; Jellesen, M. S.; Møller, P.; Frankild, S.; Johansen, J. D.; Menné, T.; Thyssen, J. P. Nickel Allergy and Dermatitis Following Use of a Laptop Computer. *J. Am. Acad. Dermatol.* **2012**, *67*, e170−e171.

(5) Flint, G. N. A Metallurgical Approach to Metal Contact Dermatitis. *Contact Dermatitis* **2007**, *39*, 213−221.

(6) Mahato, M.; Sherman, N. E.; Kiran Kumar Mudnakudu, N.; Joshi, N.; Briand, E.; Karp, J. M.; Vemula, P. K. Prevention of Metal Exposure: Chelating Agents and Barrier Creams BT. In *Metal Allergy: From Dermatitis to Implant and Device Failure*; Chen, J. K., Thyssen, J. P., Eds.; Springer International Publishing: Cham, 2018; pp 27−246.

(7) Memon, A. A.; Molokhia, M. M.; Friedmann, P. S. The Inhibitory Effects of Topical Chelating Agents and Antioxidants on Nickel-Induced Hypersensitivity Reactions. *J. Am. Acad. Dermatol.* **1994**, *30*, 560−565.

(8) Vemula, P. K.; Anderson, R. R.; Karp, J. M. Nanoparticles Reduce Nickel Allergy by Capturing Metal Ions. *Nat. Nanotechnol.* **2011**, *6*, 291.

(9) Soesman-van, W. K. A.; Van Ketel, W. G. Persistence of Patch Test Reactions to Clioquinol (Vioform) and Cross-sensitization. *Contact Dermatitis* **2018**, *6*, 455−460.

(10) Kaaber, K.; Menne, T.; Tjell, J. C.; Veien, N. Antabuse Treatment of Nickel Dermatitis. Chelation − a New Principle in the Treatment of Nickel Dermatitis. *Contact Dermatitis* **1979**, *5*, 221−228.

(11) Christensen, O. B.; Mette, K. Treatment with Disulfiram in Chronic Nickel Hand Dermatitis. *Contact Dermatitis* **2006**, *8*, 59−63.

(12) Pillai, C. K. S.; Paul, W.; Sharma, C. P. Chitin and Chitosan Polymers: Chemistry, Solubility and Fiber Formation. *Prog. Polym. Sci.* **2009**, 641−678.

(13) Shukla, S. K.; Mishra, A. K.; Arotiba, O. A.; Mamba, B. B. hitosan-Based Nanomaterials: A State-of-the-Art Review. *Int. J. Biol. Macromol.* **2013**, *59*, 46−58.

(14) Wu, F.-C.; Tseng, R.-L.; Juang, R.-S. A Review and Experimental Verification of Using Chitosan and Its Derivatives as Adsorbents for Selected Heavy Metals. *J. Environ. Manage.* **2010**, *91*, 798−806.

(15) Zhai, H.; Maibach, H. I. *Barrier Creams and Emollients BT—Irritant Dermatitis*; Chew, A.-L., Maibach, H. I., Eds.; Springer Berlin Heidelberg: Berlin, Heidelberg, 2006; pp 79−485.

(16) Debandi, M. V.; Bernal, C.; Francois, N. J. Development of Biodegradable Films Based on Chitosan/Glycerol Blends Suitable for Biomedical Applications. *J. Tissue Sci. Eng.* **2016**, 3.

(17) Thinh, N. N.; Hanh, P. T. B.; Ha, L. T. T.; Anh, L. N.; Hoang, T. V.; Hoang, V. D.; Dang, L. H.; Khoi, N. Van; Lam, T. D. Magnetic

DOI: 10.1021/acsomega.9b00326
ACS Omega 2019, 4, 5900−5903

Chitosan Nanoparticles for Removal of Cr(VI) from Aqueous Solution. *Mater. Sci. Eng., C* **2013**, *33*, 1214−1218.
(18) Karthikeyan, T.; Rajgopal, S.; Miranda, L. R. Chromium(VI) Adsorption from Aqueous Solution by Hevea Brasilinesis Sawdust Activated Carbon. *J. Hazard. Mater.* **2005**, *124*, 192−199.
(19) Wöhrl, S.; Nicole, K.; Wolfgang, H.; Margarete, F.; Werner, B.; Manfred, G.; Reinhart, J. A Cream Containing the Chelator DTPA (Diethylenetriaminepenta-acetic Acid) Can Prevent Contact Allergic Reactions to Metals. *Contact Dermatitis* **2002**, *44*, 224−228.
(20) Ou, C.-Y.; Zhang, C.-H.; Li, S.-D.; Yang, L.; Dong, J.-J.; Mo, X.-L.; Zeng, M.-T. Thermal Degradation Kinetics of Chitosan−cobalt Complex as Studied by Thermogravimetric Analysis. *Carbohydr. Polym.* **2010**, *82*, 1284−1289.
(21) Nagireddi, S.; Katiyar, V.; Uppaluri, R. Pd(II) Adsorption Characteristics of Glutaraldehyde Cross-Linked Chitosan Copolymer Resin. *Int. J. Biol. Macromol.* **2017**, *94*, 72−84.
(22) Ma, Y.; Xin, L.; Tan, H.; Fan, M.; Li, J.; Jia, Y.; Ling, Z.; Chen, Y.; Hu, X. Chitosan Membrane Dressings Toughened by Glycerol to Load Antibacterial Drugs for Wound Healing. *Mater. Sci. Eng., C* **2017**, *81*, 522−531.
(23) Hussein, M. H. M.; El-Hady, M. F.; Sayed, W. M.; Hefni, H. Preparation of Some Chitosan Heavy Metal Complexes and Study of Its Properties. *Polym. Sci., Ser. A* **2012**, *54*, 113−124.
(24) Llanos, J. H. R.; Vercik, L. C.; de, O.; Vercik, A. Physical Properties of Chitosan Films Obtained after Neutralization of Polycation by Slow Drip Method. *J. Biomater. Nanobiotechnol.* **2015**, 276−291.
(25) Tanojo, H.; Hostýnek, J. J.; Mountford, H. S.; Maibach, H. I. In Vitro Permeation of Nickel Salts through Human Stratum Corneum. *Acta Derm.-Venereol.* **2001**, *212*, 19.
(26) Larese, F.; Gianpietro, A.; Venier, M.; Maina, G.; Renzi, N. In Vitro Percutaneous Absorption of Metal Compounds. *Toxicol. Lett.* **2007**, *170*, 49−56.
(27) Fluhr, J. W.; Gloor, M.; Lehmann, L.; Lazzerini, S.; Distante, F.; Berardesca, E. Glycerol Accelerates Recovery of Barrier Function in Vivo. *Acta Derm.-Venereol.* **1999**, *79*, 418.

DOI: 10.1021/acsomega.9b00326
ACS Omega 2019, 4, 5900−5903