Mini Review

Assessment of the release of nickel from biomaterials in vivo and in vitro: enhancement by lipopolysaccharide

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Biodevices are implanted for long periods of time, so the release of metal ions from alloys should be tested in tissues to assess the risk of inducing metal allergies. However there is little evidence that the release of metal ions from alloys *in vivo* is similar to that *in vitro*. We implanted metal wires in mice and determined the concentration of metal ions in tissue to analyze the mechanisms responsible for metal allergies. The release of ions from the Ni wire was detected within 8 h and attained a plateau 72 h after the implantation. Furthermore, it was significantly increased by an injection of LPS. The results indicated that the release of Ni was apparently enhanced by inflammatory responses. We also established an *in vitro* assay system using the murine macrophage cell line RAW 264. The addition of LPS apparently increased the amount of Ni released into the medium, indicating the activation of the cells to have enhanced the elution of ions from the Ni plate. Our *in vitro* model using LPS-stimulated RAW264 cells might reflect the elution of Ni in inflamed tissue.

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Introduction

Implantations of biomedical devices to treat diseases and organ insufficiencies are increasing as populationage [1]. Most devices for the replacement of hard tissues, including artificial hip joints, bone plates and dental implants, comprise metallic biomaterials because of their reliable mechanical performance [2-3]. Corrosion-resistant and ductile[3] nickel (Ni) is contained in various alloys, including stainless steels, Ni-Cr and Ni-Ti. However, Ni is also the most common contact allergen among metals[4-5]. Allergies to Ni, classified as Type IV allergies [4-6], are initiated by the release of Ni ions from alloys and the forming of Ni-binding proteins, which are recognized as antigens by antigen-presenting cells such as dendritic cells (DC) and macrophages [4]. These cells then activate T cells and induce an increase in the number of Ni-specific, IFN-γ-producing $CD4^+$ and $CD8^+$ effector T cells[4-6]. It is difficult to prevent Ni allergies by inhibiting these immune responses. A more practical approach might be to block the elution of Ni ions from biomaterials. However, the molecular mechanisms of this elution have not been fully examined.
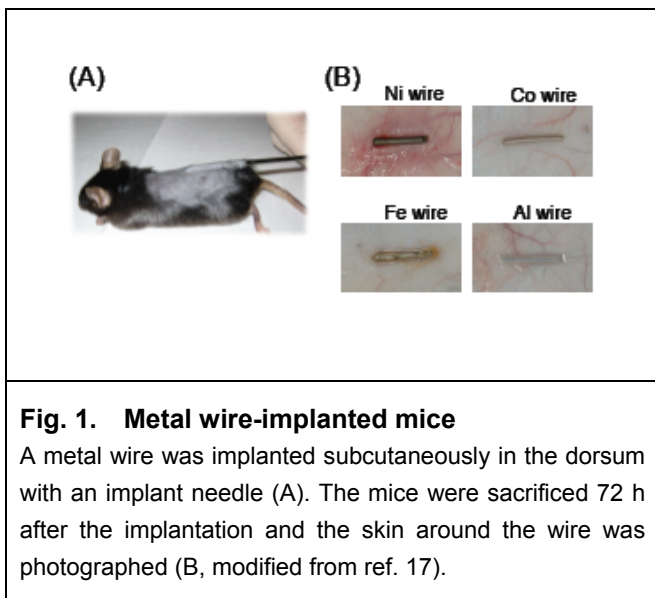

Fig. 1. Metal wire-implanted mice

A metal wire was implanted subcutaneously in the dorsum with an implant needle (A). The mice were sacrificed 72 h after the implantation and the skin around the wire was photographed (B, modified from ref. 17).

In general, the release of Ni ions from alloys is tested in solutions. For example, Okazaki *et al.* determined the release of metal ions from SUS316L stainless steel and Co–Cr–Mo casting alloy immersed in α-medium, PBS (−), calf serum, 0.9% NaCl, artificial saliva, 1.2 % L-cysteine, 1 % lactic acid and 0.01 % HCl for 7 days [7]. As biodevices are implanted for long periods of time, one should test the release of metal ions from alloys in tissues to assess the risk of inducing metal allergies. However, there is little evidence that the release of metal ions from alloys *in vivo* is similar to that *in vitro*.

Animal models of Ni-induced allergy and inflammation

To reveal the mechanisms of Ni-induced inflammation and allergy, several animal models have been developed. The injection of Ni ions into sensitized animals was found to cause allergic inflammation including ear swelling[8-10], footpad edema[12] and the proliferation of lymph node cells[8, 12]. Tolerance to nickel sensitization was also examined in these models[9, 11]. However, it is much more difficult to induce an allergy to Ni than to a proteinous antigen. In general, a high concentration of Ni or an oxidized form of the ion such as Ni (III) or Ni (IV) is required to trigger inflammation or an allergy[8]. Recently, Sato et al. found that the co-administration of lipopolysaccharide (LPS), a stimulator of innate immune responses, effectively enhanced sensitization to Ni [10]. Sensitization using Ni ions plus LPS will promote research into the evocation of Ni allergies. In addition, the toxicity of Ni particles to lung was assessed by injecting intratracheally in rats and mice[13-15]. The Ni particles induced the infiltration of leukocytes in bronchalveolar lavage fluid and production of tumor necrosis factor-α in this model[15]. Wataha *et al.* implanted a Ni wire into the subcutaneous space of rats and analyzed Ni concentrations in tissues and necrosis[16]. According to the methods described by Wataha et al., we implanted a metal subcutaneously in the dorsum of mice using an implant needle (Fig. 1A). We examined the effects of Ni and Co wires because Ni and Co were well-known metals inducing metal allergy. In addition, the Fe and Al wires were used as the control to clarify the nonspecific inflammation induced by the implantation of wires. The Ni wire caused extreme inflammation but the Co, Fe, and Al wires did not (Fig. 1B), indicating that Ni ions are easily released and cause inflammation[17]. In this model, the Ni wire-induced inflammation was assessed as the increase in vascular permeability, which was determined by the leakage of Evans blue[17]. The implantation of Ni wire also induced the expression of cyclooxygease-2 and histidine decarboxylase in the surrounding tissues[17].

Quantitative analysis of ions release from the implanted Ni wire

Analytical techniques like inductively coupled plasma-atomic emission spectrometry (ICP-AES) and ICP-Mass spectrometry (MS) are used for the detection of metals in solution. Wataha *et al.* analyzed Ni concentrations in tissues around the Ni wire 48 h, 96 h and 7 days later using laser-ablation (LA)-ICP-MS [16]. They found that the Ni caused severe inflammation and necrosis, and that its distribution in tissues correlated well with the inflammation. They appeared that greater than 25 μg/g of Ni in tissue was necessary to elicit severe inflammation with necrosis. In *in vitro* experiments using fibroblasts, endothelial cells, and monocytes, 10-50 μg/ml of Ni ions cause total suppression of mitochondrial function [18-20]. However, the concentration at which Ni ions induce Ni allergy has not been determined. Therefore, the quantitative analysis of the release of Ni ions from materials *in vivo* is required to assess the safety of biomaterials. We developed a fluorometric assay to determine Ni concentrations using Newport Green. Thierse et al. examined the binding of Ni to membranes of human Raji B cells by flow cytometry with Newport Green [21]. The use of Newport Green will reveal where Ni binds to protein as well as its concentration in tissues. Using the Ni wire-implanted mice described above, we determined the concentration of Ni in tissue and found that the release of Ni ions was detectable within 8 h and attained a plateau 72 h after the implantation. Vascular permeability around the wire also increased from 8 h post-implantation [16]. Interestingly, when the wire was incubated at 37 °C for 8 h in water, saline, or mouse serum, very little Ni was released. Thus, Ni ions were released more easily *in vivo* than in the solutions, indicating the release to be highly dependent on the responses of cells around the wire.

Enhancement by lipopolysaccharide of the release of Ni ions *in vivo*

The implantation of biomedical devices can cause infections and bacteria-induced inflammation. Although the inflamed sites become acidic, it is not clear whether the infection and inflammation affect the elution of Ni. So we examined the involvement of inflammatory responses in the release of Ni *in vivo*. LPS (1 μg/ 200 μL saline) was injected subcutaneously around the wire at the time of implantation. The release of Ni ions into tissue was significantly increased by the injection of LPS compared to an injection of the vehicle only. These results clearly indicated that the release of Ni was enhanced by the inflammatory responses. Thus, the elution of metal ions was induced by the attached cells on the surface of alloys and enhanced by inflammatory stimuli. These findings suggested that the release of metal ions from biomaterials in buffers might not reflect that in tissues. Thus, our metal-wire implant model might be used to assess the safety of biomaterials in normal tissues and in the inflammatory conditions.
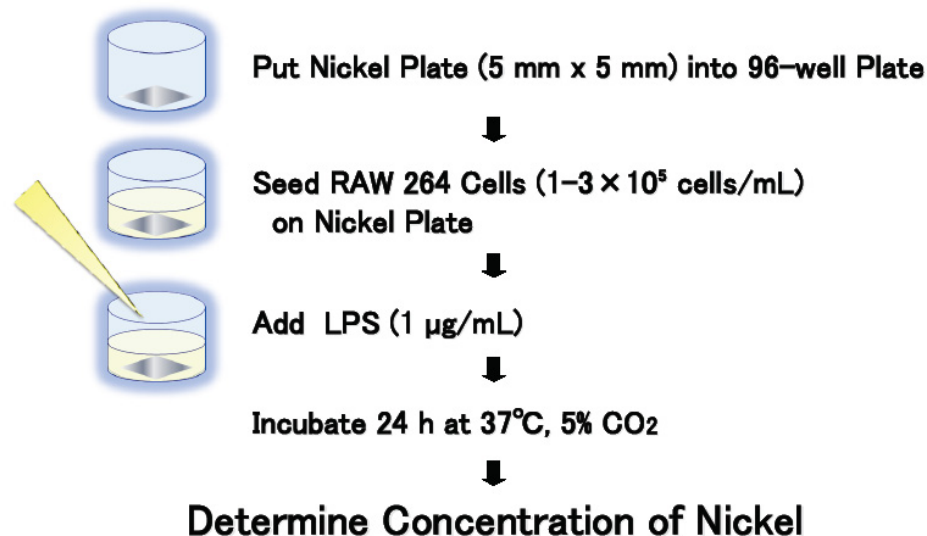
Put Nickel Plate (5 mm x 5 mm) into 96−well Plate

↓

Seed RAW 264 Cells ($1-3 \times 10^5$ cells/mL) on Nickel Plate

↓

Add LPS (1 μg/mL)

↓

Incubate 24 h at 37°C, 5% CO_2

↓

Determine Concentration of Nickel

Fig. 2. *In vitro* assay system for Ni release from the plate

In vitro system for the assessment of Ni release at inflamed sites

The effects of inflammatory responses to infections on the release of Ni from alloys have not been analyzed *in vitro*. The finding that LPS enhanced the release of ions from a Ni wire implanted in mice, however, indicates that the corrosion of metals should be tested under condition similar to those in inflamed tissue. Consequently, we established an *in vitro* assay system using the murine macrophage cell line RAW 264 as the inflammatory cells activated by LPS (Fig. 2). RAW 264 cells were seeded on a Ni plate (5 mm square) and incubated for 4, 8 and 24 h in the presence or absence of LPS. Although little Ni was released from the plate in the medium alone, a significant increase occurred in the presence of the cells. LPS (0.1 to 1 µg/mL) significantly enhanced the release of Ni into the medium in concentration- and time-dependent manners, indicating that the activation of RAW 264 cells enhanced the elution of ions from the plate. These findings suggested our *in vitro* model using LPS-stimulated RAW264 cells to be a novel system reflecting the elution of Ni at the site of inflammation.

Our findings indicated that the interaction of the cells with metals on its surface is important to release of metal ions. However, it has not been disclosed that the molecular mechanisms by which activated inflammatory cells elute metal ions from the surface of metals. Our *in vitro* model using macrophages is useful to analyze the mechanisms.

Conclusion

Metal allergy is a concern when using biomedical devices containing metals. The most effective way to avoid it is to block the release of metal ions from biomaterials. However, assay systems had not been able to assess the corrosion of metals under inflammatory conditions. We established *in vivo* and *in vitro* models for assessing the release of Ni from metal. In these models, the activation of cells such as macrophages apparently enhanced the corrosion of Ni. Thus, to assess the safety of biomaterials, one should evaluate the release of metal ions under inflammatory conditions. Our models might be useful tools for this purpose. We employed Ni wires and plates to maximize the release of Ni. If using ICP-APS and ICP-MS within our models, the release of various metal ions from biomedical alloys could be assessed at the same time.

References

1) Lee K, Goodman SB: Current state and future of joint replacements in the hip and knee. Expert Rev Med Devices. 2008; 5: 383-393.

2) Gotman I: Characteristics of metals used in implants. J Endocrinol. 1997; 11: 383-389.

3) Niinomi M: Metallic biomaterials. J Artif Organs. 2008; 11: 105-110.

4) Basketter DA, Briatico-Vangosa G, Kaestner W, Lally C, Bontinck WJ: Nickel, cobalt and chromium in consumer products: a role in allergic contact dermatitis? Contact Dermatitis. 1993; 28: 15-25.

5) Czarnobilska E, Obtułowicz K, Wsołek K, Pietowska J, Spiewak R: Mechanisms of nickel allergy. Przegl Lek. 2007; 64: 502-505.

6) Thierse HJ, Gamerdinger K, Junkes C, Guerreiro N, Weltzien HU: T cell receptor (TCR) interaction with haptens: metal ions as non-classical haptens. Toxicology. 2005; 209: 101-107.

7) Okazaki Y, Gotoh E: Comparison of metal release from various metallic biomaterials in vitro. Biomaterials. 2005; 26: 11-21.

8) Artik S, von Vultée C, Gleichmann E, Schwarz T, Griem P: Nickel allergy in mice: enhanced sensitization capacity of nickel at higher oxidation states. J Immunol. 1999; 163: 1143-1152.

9) Artik S, Haarhuis K, Wu X, Begerow J, Gleichmann E: Tolerance to nickel: oral nickel administration induces a high frequency of anergic T cells with persistent suppressor activity. J Immunol. 2001; 167: 6794-6803.

10) Sato N, Kinbara M, Kuroishi T, Kimura K, Iwakura Y, Ohtsu H, Sugawara S, Endo Y: Lipopolysaccharide promotes and augments metal allergies in mice,dependent on innate immunity and histidine decarboxylase. Clin Exp Allergy 2007; 37: 743-751.

11) Ishii N, Moriguchi N, Nakajima H, Tanaka S, Amemiya F: Nickel sulfate-specific suppressor T cells induced by nickel sulfate in drinking water. J Dermatol Sci.1993; 6: 159-164.

12) Ishii N, Sugita Y, Nakajima H, Tanaka S, Askenase PW: Elicitation of nickel sulfate (NiSO4)-specific delayed-type hypersensitivity requires early-occurring and early-acting, NiSO4-specific DTH-initiating cells with an unusual mixed pheno-

type for an antigen-specific cell. Cell Immunol. 1995; 161: 244-255.

13) Benson, JM, Burt, RL, Carpenter, RL, Edison, AF, Hahn, FF, Haley, PJ, Hanson, PJ, Hanson, RL, Hobbs, CH, Pickrell, JA, Dunnick, JK: Comarative inhalation toxicity of nickel sulfate to F344/N rats and B6C3F1 mice exposed for 12 days. Fundam Appl Toxicol. 1988; 9: 252-265.

14) Benson JM, Burt, RL, Cheng YS, Hahn, FF, Haley, PJ, Henderson, RF, Hobbs, CH, Pickrell, JA, Dunnick, JK: Biochemical responses of rats and mouse lung to inhaled nickel compounds. Toxicol. 1989; 57: 255-266.

15) Zhang, Q, Kusama, Y, Zhu, X, Sato, K, Mo, Y, Kluz, T, Donaldson, K: Comparative toxicity of standard nickel and ultrafine nickel in lung after intratracheal instillation. J. Occup Health. 2003; 45: 23-30.

16) Wataha JC, O'Dell NL, Singh BB, Ghazi M, Whitford GM, Lockwood PE: Relating nickel-induced tissue inflammation to nickel release in vivo. J Biomed Mater Res. 2001; 58: 537-544.

17) Hirasawa N, Goi Y, Tanaka R, Ishihara K, Ohtsu H, Ohuchi K: Involvement of prostaglandins and histamine in nickel wire-induced acute inflammation in mice. J Biomed Mater Res A. 2010; 93: 1306-1311.

18) Wataha JC, Hanks CT, Craig RG: The in vitro effects of metal cations on eulariotic cell metabolism. J Biomed Mater Res. 1991; 25: 1133-1149.

19) Wataha JC, Hanks CT, Sun ZL: In vitro reaction of macrophages to metal ions from dental biomaterials. Dent Mater. 1995; 11: 239-245.

20) Wataha JC, Sun ZL, Hanks CT, Fang DN: Effects of Ni ions on expression of intercellular adhesion molecule 1 by endothelial cells. J Biomed Mater Res. 1997; 36: 145-151.

21) Thierse HJ, Helm S, Pink M, Weltzien H: Novel fluorescence assay for tracking molecular and cellular allergen-protein interactions. J Immunol Methods. 2007; 328: 14-20.