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A comparative study of allergic contact dermatitis by patch test *versus* reflectance confocal laser microscopy, with nickel and cobalt

Few studies have reported on the accuracy of reflectance confocal laser microscopy (RCLM) in observing allergic contact dermatitis *in vivo*. However, distinction of skin reactions from different reagents is not well understood. We sought to diagnose allergic contact dermatitis by RCLM images and compare with routine, visual patch test (PT) reading for 2 major allergen metals in Japan. The PT was performed on the upper back skin with 5% nickel sulfate (Ni) and 2% cobalt chloride (Co) in eight healthy volunteers and eleven patients. RCLM was used to calculate the thickness of the suprabasal epidermis after visual assessment of PT. Comparison of clinical scoring versus suprabasal epidermal thickness was observed. RCLM images of positive PT showed increased suprabasal epidermal thickness on day 2 (D2), and D3 for Co, whereas there was vesicle formation and an overall increase suprabasal epidermal thickness for Ni. In two of 3 doubtful positive PT to Co, RCLM images presented characteristics of irritant reactions; and one characteristic of a positive reaction. The frequency of Co doubtful-positive PT was higher than that of Ni. We found advantages in using RCLM for visualizing features of allergic contact dermatitis and found it a useful tool as an objective parameter in grading severity and types of PT reaction.

Key words: allergic contact dermatitis, dental alloy, metal allergy, patch test, reflectance confocal laser microscopy, suprabasal epidermis

Article accepted on 9/6/2010

Traditionally, different types of alloys have been used in restorative dentistry. The common criterion for all these materials is their permanent existence in the oral cavity for a prolonged period of time and this exposure may sensitize patients. The clinical manifestations of contact allergy to dental alloy are not uniform. Diseases such as pustulosis palmaris et plantaris, lichen planus, systemic or palmoplantar eczema, symptoms like glossodynia, cheilitis related with ions released from these metals are well documented [1-6]. Furthermore, the Japanese Ministry of Health and Welfare reported in 1997 that allergy affects approximately 30% of the population in Japan and recently, the frequency of dental metal allergy has risen significantly [7-9].

Patch testing is the primary tool to diagnose allergens causing allergic contact dermatitis. On the other hand, this method is strongly dependent on the experience of

the observer, and distinguishing doubtful-positive from positive patch test (PT) reactions for different reagents remains difficult [10-14]. Reflectance confocal laser microscopy (RCLM) has been used to image human skin non-invasively, providing a virtual window into tissues *in vivo* without staining processes or destruction of the skin [15-18]. More recently, diagnostic criteria with RCLM were investigated to characterize features of allergic contact dermatitis. These criteria include vesicle formation, inflammatory infiltrate, spongiosis and exocytosis [19-22]. However, skin reactions to different metal allergens and changes in epidermal thickness are still not well understood [23, 24]. The aim of this study was to compare suprabasal epidermal thickness, using RCLM, with clinical grading of PT from two major allergen metals *i.e.*, nickel (Ni) and cobalt (Co) in Japan.

Materials and methods

Subjects

Eight healthy volunteers (3 males and 5 females, mean age 30 years), and eleven patients (1 male and 10 females, mean age 47 years) with suspected contact allergy to dental metals and confirmed presence of dental metal restora-

Abbreviations:

Co	2% cobalt chloride
D	day
Ni	5% nickel sulfate
PT	patch test
RCLM	reflectance confocal laser microscopy

Table 1. Characteristics of patients

Patient	Age/ Sex	Symptoms & systemic diseases	Years of symptoms & systemic diseases	Dental metal restorations	Mean years in use dental metal restoration
1	36/F	CD (jewellery)	20	Au-Ag-Pd alloy	10
2	67/F	CD (jewellery, leather)	6	Au-Ag-Pd alloy	20
		HF	5		
3	28/F	eczema (face, neck, head)	1 month	Au-Ag-Pd alloy	8
		HF, sinusitis	8		
4	38/F	CD (jewellery)	15	Amalgam	22
		stomatitis	2	Au-Ag-Pd alloy	10
5	67/F	CD (jewellery)	17	Au-Ag-Pd alloy	40
		adenoiditis	50	Co-Cr alloy	20
		loxoprofen allergy	7		
6	58/F	stomatitis	1	Au-Ag-Pd alloy	25
7	68/F	oral lichen planus on buccal surface adjacent to the metal restoration	2	Au-Ag-Pd alloy	40
8	40/M	pustulosis palmaris et plantaris	8 months	Au-Ag-Pd alloy	20
9	66/F	CD (jewellery)	26	Au-Ag-Pd alloy	41
10	51/F	CD (jewellery)	10	Au-Ag-Pd alloy	20
				Ni-Cr alloy	30
11	44/F	cheilitis facial erythema	2	Au-Ag-Pd alloy	20
				Co-Cr alloy	2

CD: contact dermatitis; HF: hay fever; Au-Ag-Pd: gold, silver, palladium, Copper, tin, zinc, and silicon alloy; Co-Cr: cobalt, chromium, molybdenum, and nickel alloy; Ni-Cr: nickel, chromium, iron, and silicone alloy.

tion from the Dental Allergy Clinic of Tokyo Medical and Dental University were recruited for this study (table 1). We received approval from the Dental Research Ethics Committee of Tokyo Medical and Dental University and informed consent was obtained from all contributors.

Exposure to reagents

The upper back skin was exposed to aqueous solutions of 5% nickel sulfate (Ni) and 2% cobalt chloride (Co) (Torii Pharmaceutical Co. Ltd, Tokyo, Japan) using patch tester TORII® (9-mm diameter, Torii Pharmaceutical Co. Ltd, Tokyo, Japan). The testers were removed by the subject after 48 h on day 2 (D2).

Evaluation

Clinical evaluation

Skin readings were performed 20 min after removal of the testers on D2, D3, and, D7 according to the clinical scoring criteria recommended by the International Contact Dermatitis Research Group [21] (table 2). Positive reactions on D3 or D7 were judged as allergic contact sensitivity. Skin reaction only on D2 and faint erythema or absence of reaction on D3 and/or D7 was judged as doubtful-positive reaction. In addition, clinical photographs of skin reactions were taken under standardized conditions with a digital camera (Nikon Coolpix 950, Nikon Corp, Tokyo, Japan).

RCLM evaluation

A commercially available RCLM (Vivascope 1500 Plus, Lucid Inc, Henrietta, NY) was used to produce horizontal (surface) images of skin sites with X, Y, and vertical (in depth) images with Z plane micrometer screws. This

device use a diode laser at 830 nm with a power less than 16 mW at tissue level. The X30 water-immersion lens of numerical aperture 0.9 was applied to the skin. It was immobilized with a tissue ring and template fixture device to provide standardized mechanical contact with the RCLM. Details of the instrument are given elsewhere [16, 17]. In each of the skin sites analyzed, a systematic 4 mm² X-Y mapping was performed and 5 images were captured in Z plane per 1µm in depth, beginning at the stratum corneum and going through the epidermis and into the upper reticular dermis. The suprabasal epidermis (from the surface of the stratum corneum with presence of corneocytes to the bottom of the cells in the uppermost portion of the stratum basale) were used to calculate thicknesses before (D0) and after testers were removed (D2, D3, D7) (figure 1, A panel).

Statistical analysis

Suprabasal epidermal thickness of healthy volunteers and patients before PT was analyzed using the Student t-test.

Table 2. Criteria for clinical scoring of allergic contact dermatitis reactions

Score	Allergic contact dermatitis
0	Negative
0.5	Barely erythema
1	Erythema, infiltration, possibly papules
2	Erythema, infiltration, papules, vesicles
3	Intensive erythema, bullous reaction or erosion

The scheme follows the guidelines of the International Contact Dermatitis Research Group.

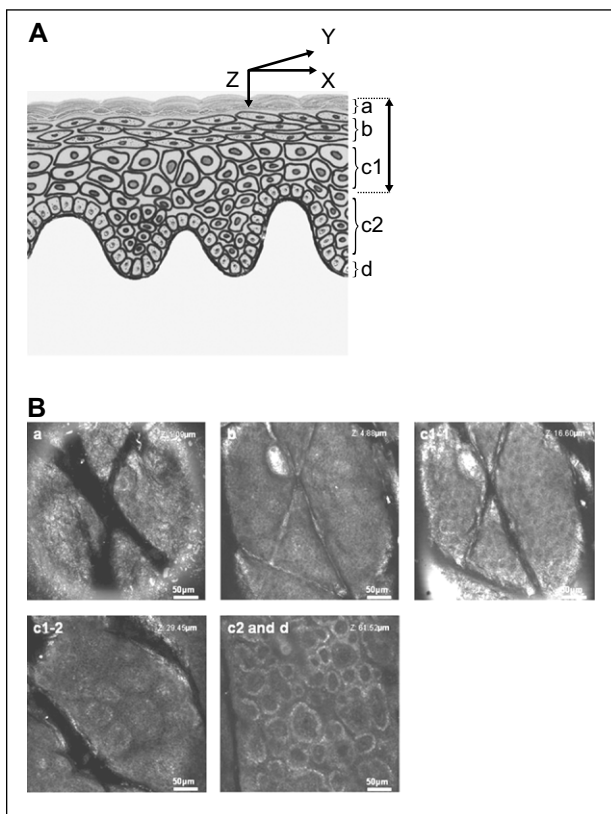


Figure 1. **A panel**, Vertical section in XZ planes of the skin illustration and **B panel**, horizontal section in XY planes of the RCLM images. **a.** stratum corneum **b.** stratum granulosum **c1.** stratum supinosum above papillary dermis (in RCLM images, **c1-1.** upper and, **c1-2.** lower layer) **c2.** stratum supinosum between papillary dermis **d.** stratum basale (in RCLM images, **c2 and d.**). The arrow indicates the suprabasal epidermis (from the surface of the stratum corneum to the bottom of the cells in the uppermost portion of the stratum basale). The mean thickness from 5 RCLM images observed in this subject: 21, 92 µm SD ± 4.37.

Changes of suprabasal epidermal thickness at the time points of skin readings were analyzed using one-way ANOVA and Tukey HSD test [25, 26]. Furthermore, in patients, the paired t-test was used on the same subjects to analyze differences in suprabasal epidermal thickness between Ni and Co. Clinical scores were performed with the Mann-Whitney U test and Regression analyses to determine the correlations between suprabasal epidermal thickness and elapsed period of days [27]. All analyses were conducted using SPSS Ver.12.0J for Windows. For global significance, P less than 0.05 and for Bonferroni, P less than 0.016 was considered statistically significant.

Results

Clinical evaluation

Healthy volunteers

All subjects were found to be clinically negative to Ni and Co. However, punctate erythema and hemorrhagic around the hair follicles openings, presenting small dots,

irregularly distributed within the test area were observed on D2 and D3 for Co on two of 8 healthy volunteers (figures 2, A and B panel).

Patients

From 11 subjects three were found to be doubtful-positive PT to Co, two were positive PT to Co and four were positive PT to Ni. ($P < 0.05$ and $P < 0.016$) (figures 2, C and D panel; tables 3, 4).

RCLM evaluation

Suprabasal epidermal thickness did not show a significant difference between healthy volunteers and patients on D0 ($P > 0.05$) (figure 1 B panel) [18].

Healthy volunteers

All subjects showed an increasing tendency of suprabasal epidermal thickness on D2, decreasing consequently until normal parameters on D7 (figure 3). Statistical analysis showed no significant differences among time points of assessment for Ni and Co, nor between the reagents ($P > 0.05$).

Patients

The increasing suprabasal epidermal thickness on Ni and Co was greater in patients than healthy volunteers ($t = -5.942$, $P < 0.0001$). We found statistical differences between D0 and D2, D0 and D3, D0 and D7 for Ni and Co respectively ($P < 0.05$). Between the reagents, statistical significance showed on D2 and D7 ($t = 2.094$, $P = 0.049$) ($t = 0.692$, $P = 0.0497$). For each PT result, the average suprabasal epidermal thicknesses from D2, D3, and D7; Ni-positive and Ni-negative, Co-positive and Co-negative, Co-doubtful-positive and Co-negative were statistically significant ($P < 0.05$ and $P < 0.016$) (figure 4, table 4).

Regression analyses of suprabasal epidermal thickness in positive reactions to Ni vs. elapsed period by days were significantly strong positive ($R^2 = 0.6191$, $P < 0.0003$), and negative Ni reactions showed a significantly moderate positive correlation ($R^2 = 0.3119$, $P < 0.0071$) (figures 5A, B). For Co, regression analysis of suprabasal epidermal thickness vs. elapsed period by days showed moderate positive significance in positive Co reactions ($R^2 = 0.5686$, $P = 0.0322$), strong positive significant correlation in negative Co reactions ($R^2 = 0.6993$, $P < 0.009$) and not significant correlation in doubtful-positive Co reactions ($P = 0.0535$) (figures 5C, D, E).

Discussion

PT is said to be the most conventional evaluation method for allergic contact dermatitis [28]. The instrument used for clinical assessment is a combination of vision and feel in the form of palpation with the examiner's finger, and this measurement is a totally subjective method based on the clinician's knowledge and experience.

Interestingly, previous reports of PT readings have shown the disagreement on scoring among examiners under the same conditions [29]. The RCLM represents a useful measurement for determining an individual's skin hypersensitivity [30, 31].

In our study, we evaluated the efficiency of the RCLM in grading the severity of allergic skin reactions to Ni and Co and determined the correlation with routine visual PT

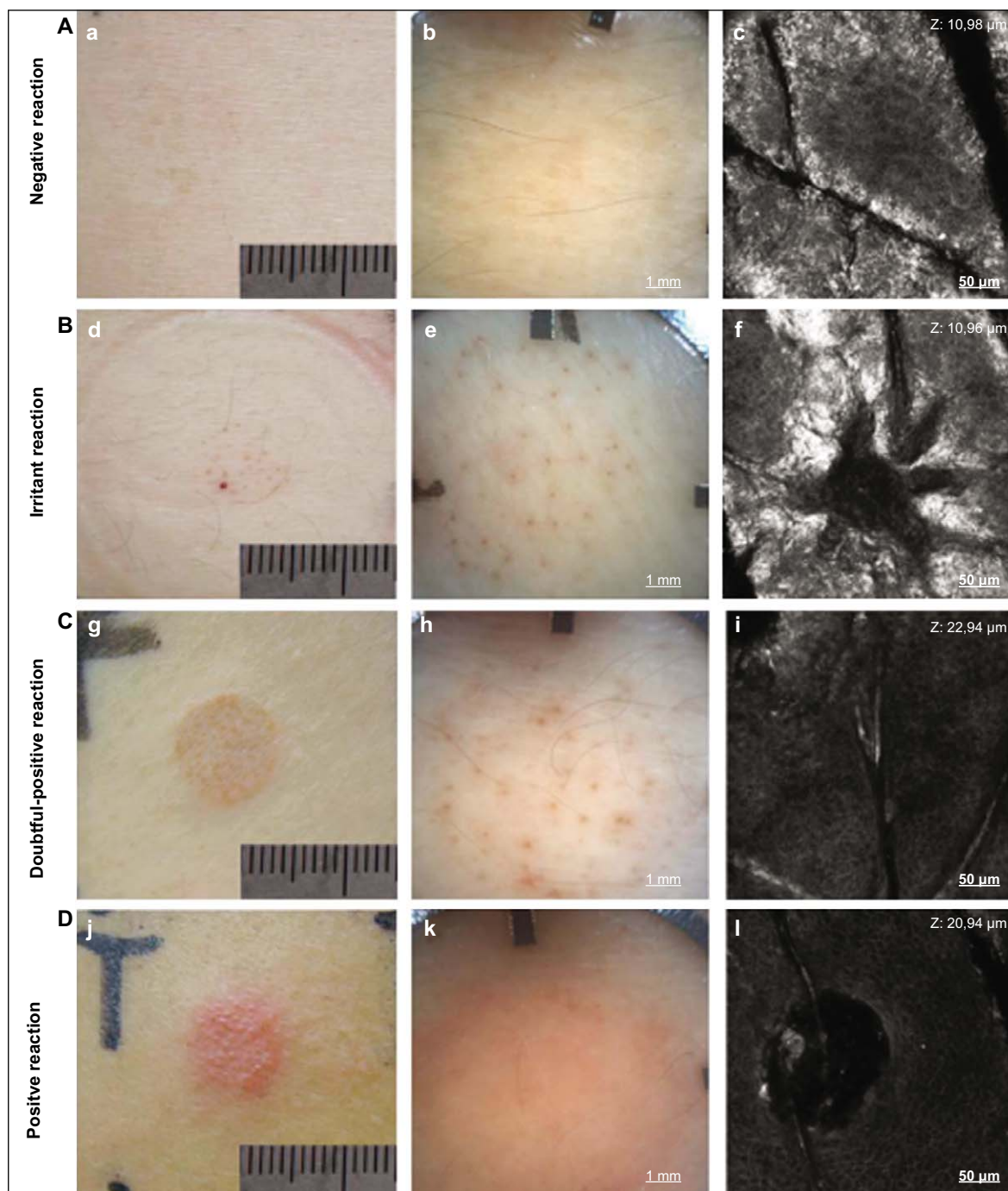


Figure 2. Clinical images and corresponding RCLM images at D3 are shown. **A panel**, Images (a) through (c): **a, b**: clinical images of negative reaction **c**: RCLM evaluation shows stratum corneum **B panel**, Images (d) through (f): **d, e**: clinical images of irritant reaction with punctate erythema and slightly hemorrhagic around hair follicle openings **f**: RCLM evaluation shows disruption of stratum corneum. **C panel**, Images (g) through (i): **g, h**: clinical images of doubtful positive reaction, **i**: RCLM evaluation shows spongiosis in stratum supinosum. **D panel**, Images (j) through (k): **j, k**: clinical images of positive reaction, **l**: RCLM evaluation shows vesicle formation in stratum supinosum.

results. Patients showed a significant increase in suprabasal epidermal thickness compared with healthy volunteers. This indicated that the differences observed on the skin response could be explained by the occlusive effect and the metal reagents of the patch tester acting as mechanical stimuli with the additional response of specific T-cell involvement in patients [32].

The Ni-positive group showed an overall increase in suprabasal epidermal thickness, intercellular edema, acanthosis and great vesicle formation. This result might be due to a stronger proliferative response of keratinocytes and T-cell involvement in the pathogenesis of metal hypersensitivity than that to Co [19, 33, 34]. The Co-positive group showed an increase of the suprabasal

Table 3. Severity of clinical scoring in patch test for Ni and Co

Patient	Clinical scores	
	Ni	Co
1	2	0
2	2	0
3	1	0
4	1	0
5	0	0
6	0	0.5
7	0	0
8	0	1
9	0	0.5
10	0	1
11	0	0.5

Ni: 5% nickel sulfate; Co: 2% cobalt chloride.

epidermal thickness mainly on D2 and D3, and both positive groups were well correlated with respective clinical aspects of PT scoring in our study.

In Co-doubtful positive group, the RCLM images showed two different aspects.

– The presence of superficial disruption in the stratum corneum on two of 3 patients. However, we did not observe RCLM morphologic features common to allergic contact dermatitis [24, 29, 35].

– The increase of suprabasal epidermal thickness in the remaining one of the 3 patients, which could be recognized as positive in RCLM, the visual assessment by PT displayed doubtful positive. The facial erythema and cheilitis of this patient were improved after the removal of a cobalt-chrome metal frame-removable denture with no additional treatment.

Moreover, we found that Co showed more doubtful-positive PT reactions than Ni (Ni-positive: 21.44%, Ni doubtful-positive: 5.15%, Co-positive: 10.72%, Co doubtful-positive: 7.01%, ($\chi^2 = 10.527$, Haberman's test, $p < 0.002$) from 485 patients (aged between 27 and 82 years, mean age 48 years) who visited our clinic from 2006 to 2007 (data not shown) [36]. This result supported previous studies that the characteristics of allergic reactions in Co showed weaker signs compared to those of the Ni group [23, 24, 37, 38].

Table 4. Percentage distribution of clinical scores and average of suprabasal epidermal thickness from D2, D3, D7 among Ni and Co in patients

Reagent	Patch test result	Clinical Scores median (25th/75th)	Average on suprabasal epidermal thickness from D2, D3, D7 mean (SD) μm
Ni	positive ($n = 4$)	1 (1/2) a*	37.25 (8.14) a*
	negative ($n = 7$)	0 (0/0) a*	25.33 (5.92) a*
Co	positive ($n = 2$)	1 (1/1) b1*	30.83 (6.7) b1*
	doubtful positive ($n = 3$)	0.5 (0.5/0.5) b2*	30 (4.97) b2*
	negative ($n = 6$)	0 (0/0) b1* b2*	24.06 (2.38) b1* b2*

Ni: 5% nickel sulfate; Co: 2% cobalt chloride.

Values of clinical scores are median and percentiles (25th/75th).

Mann-Whitney U test and Student t-test shown statistically significant (a*: $p < 0.05$) (b*: $p < 0.016$).

b1*: statistical significant between Co positive and negative.

b2*: statistical significant between Co doubtful positive and negative.

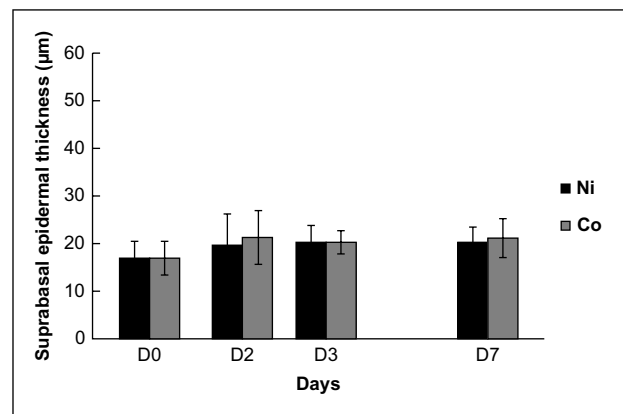


Figure 3. Suprabasal epidermal thickness in healthy volunteers with 5% nickel sulfate and 2% cobalt chloride before (D0) and after removed the testers (D2, D3, and D7). Values expressed as mean $\pm$ SD.

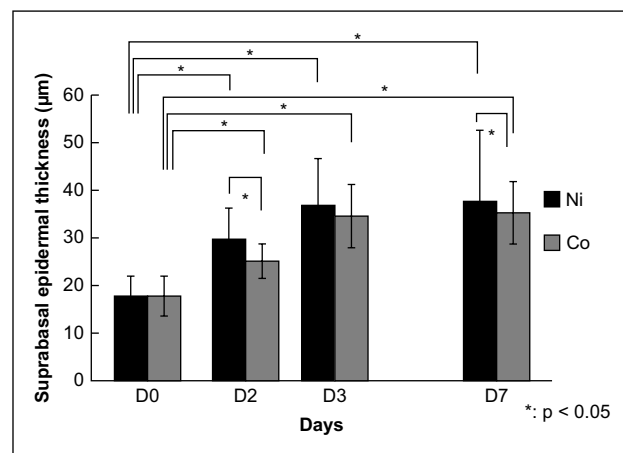


Figure 4. Suprabasal epidermal thickness in patients with 5% nickel sulfate and 2% cobalt chloride before (D0) and after removed the testers (D2, D3, and D7). Values expressed as mean $\pm$ SD.

Although we assessed only two specific allergens, Ni and Co, the morphology of PT reactions can vary with different metals. For instance, palladium and gold have a tendency to appear as a skin reaction 5 days after removing

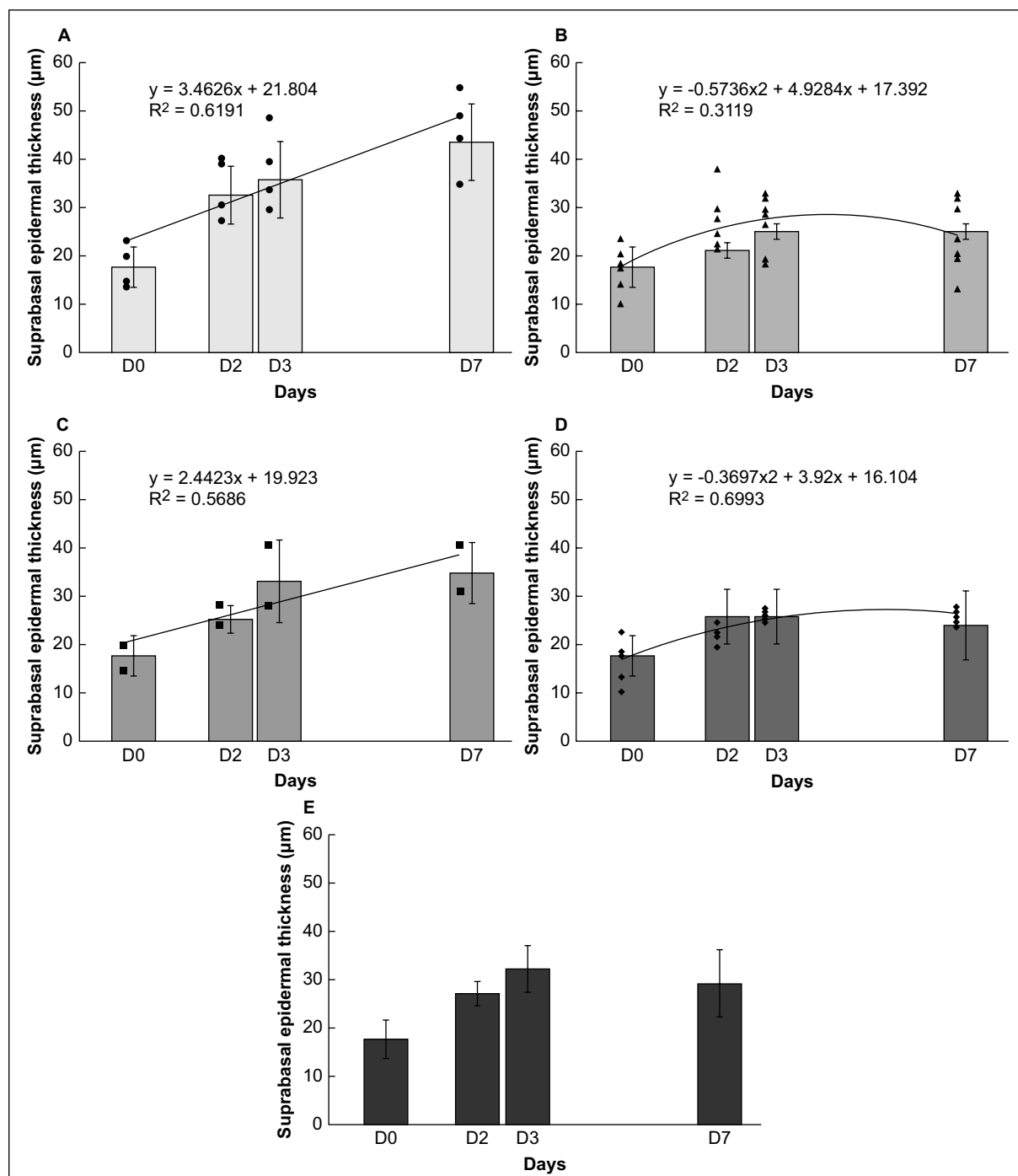


Figure 5. Regression analysis of suprabasal epidermal thickness *versus* elapsed period by days on each PT results in patients. **A)** Nickel positive reaction. **B)** Nickel negative reaction. **C)** Cobalt positive reaction. **D)** Cobalt negative reaction. **E)** Cobalt doubtful-positive reaction.

the reagent [39]. Therefore, the observations of epidermal thickness and morphological changes by the RCLM should each be performed after the visual assessment. One of the main limitations of the RCLM technique is the fundamental inability to image deep objects in the dermis. In addition, acanthosis due to intercellular edema, vesicle formation and keratinocyte proliferation can also restrict the visualization. In some other studies different concentrations of allergen were used, different types of vehicles

and other types of patch testers, for example T.R.U.E. test (Thin-layer Rapid Use Epicutaneous Test, Allerderm Lab, Phoenix, Ariz.) [40, 41]. Since different approaches and materials could lead to variations in the results, the selection criteria of the tests should be considered and structured to obtain more accurate results of the reaction processes of the allergic contact dermatitis.

Co and Ni are components of many types of alloy that can be found in daily life. However, in dental treatment, it is

possible to avoid such allergens by proper selection of materials or by using metal free restorations [42, 43]. In conclusion, our study demonstrated the potential of the RCLM to assist in more accurate interpretation of test results between allergic, doubtful and irritant reaction on each allergen, rather than by using visual assessment alone. ■

Disclosure. Acknowledgements: we would like to express gratitude to Mr. Hoshiaki Sawata to gives us insightful comments and great help during preparation of this paper. Financial support: none. Conflict of interest: none.

References

1. Fleischmann P. Zur Frage der Gefährlichkeit Kleinsten Quecksilbermenger. *DiMedWschr* 1928; 54: 304-7.
2. Nakayama H. 2 clinical cases of oral lichen planus probably related with dental metal allergy. *Otolaryngology* 1972; 44: 239-47.
3. Schmalz G, Garhammer O. Biological interactions of dental cast alloys with oral tissues. *Dent Mater* 2002; 18: 396-406.
4. Kosugi M, Ishihara K, Okuda K. Implication of responses to bacterial heat shock proteins, chronic microbial infections, and dental metal allergy in patients with pustulosis palmaris et plantaris. *Bull Tokyo Dent Coll* 2003; 44: 149-58.
5. Nakayama H. New aspects of metal allergy. *Acta Dermatovenereol Croat* 2002; 10: 207-19.
6. Lomaga MA, Polak S, Grushka M, Walsh S. Results of patch testing in patients diagnosed with oral lichen planus. *J Cutan Med Surg* 2009; 13: 88-95.
7. Evaluation of Year 2004 research operation Available at: <http://www.mhlw.go.jp/shingi/2004/10/s1006-6c06.html#11> Accessed April 15, 2009
8. Fisher AA. The role of the patch testing. In: *Contact Dermatitis*. Philadelphia: Lea and Febiger, 1986: 9-29.
9. Suzuki N. Metal Allergy in Dentistry: Detection of allergen metals with X-ray fluorescence spectroscope and its application toward allergen elimination. *Int J Prosthodont* 1995; 8: 351-69.
10. Nosbaum A, Vocanson M, Rozieres A, Hennino A, Nicolas JF. Allergic and irritant contact dermatitis. Pathophysiology and immunological diagnosis. *Eur J Dermatol* 2009; 19: 325-32.
11. Nethercott JR. Practical problems in the use of patch testing in the evaluation of patients with contact dermatitis. In: Weston WL, Mackie RM, Provost TT, eds. *Current Problems in Dermatology*. St. Louis, MO: Mosby, 1990: 101-3.
12. Nethercott JR. Sensitivity and specificity of patch tests. *Am J Contact Dermatitis* 1994; 5: 136-42.
13. Brasch J, Henseler T, Aberer W, Bauerle G, Frosch PJ, Fuchs T, et al. Reproducibility of patch tests. A multicenter study of synchronous left- versus right-sided patch tests by the German Contact Dermatitis Research Group. *J Am Acad Dermatol* 1994; 31: 584-91.
14. Sarma N. Late reaction, persistent reaction and doubtful allergic reaction: The problems of interpretation. *Indian J Dermatol* 2009; 54: 56-8.
15. Uter W, Frosch PJ, Becker D, Schnuch A, Pfahlberg A, Gefeller O. Are we biased when reading a doubtful patch test reaction to a 'clear-cut' allergen such as the thiuram mix? *Contact Dermatitis* 2009; 60: 234-5.
16. Rajadhyaksha M, Grossman M, Esterowitz D, Webb RH, Anderson RR. In vivo confocal scanning laser microscopy of human skin: melanin provides strong contrast. *J Invest Dermatol* 1995; 104: 946-52.
17. Rajadhyaksha M, Gonzalez S, Zavislan JM, Anderson RR, Webb RH. In vivo confocal scanning laser microscopy of human skin II: advances in instrumentation and comparison with histology. *J Invest Dermatol* 1999; 113: 293-303.
18. Huzaira M, Rius F, Rajadhyaksha M, Anderson RR, Gonzalez S. Topographic variations in normal skin, as viewed by in vivo reflectance confocal microscopy. *J Invest Dermatol* 2001; 116: 846-52.
19. Astner S, Gonzalez E, Cheung A, Ruiz-Diaz F, Gonzalez S. Pilot study on the sensitivity and specificity of in vivo reflectance confocal microscopy in the diagnosis of allergic contact dermatitis. *J Am Acad Dermatol* 2005; 53: 986-92.
20. Astner S, Gonzalez E, Chueung A, Ruiz-Diaz F, Doukas A, William F, et al. Non-Invasive Evaluation of the Kinetics of Allergic and Irritant Contact Dermatitis. *J Invest Dermatol* 2005; 124: 351-9.
21. Swindells K, Burnett N, Ruiz-Diaz F, Gonzalez E, Mihm MC, Gonzalez S. Reflectance confocal microscopy may differentiate acute allergic and irritant contact dermatitis in vivo. *J Am Acad Dermatol* 2004; 50: 220-8.
22. Burnett N. Confocal Microscopy Helps Distinguish between Allergic and Irritant Contact Dermatitis. *eSkin & Allergy News* 2003; 34
23. Storrs FJ, White CR. False-positive "poral" cobalt patch test reactions reside in the eccrine acrosyringium. *Cutis* 2000; 65: 49-53.
24. Fischer T, Rystedt I. False-positive, follicular and irritant patch test reactions to metal salts. *Contact Derm* 1985; 12: 93-8.
25. Snedecor GW, Cochran WG. One-way classifications. Analysis of variance. In: *Statistical Methods*. GW Snedecor and WG Cochran (eds). The Iowa State University Press, Ames, 1967: 258-298
26. Tukey JW. Some selected quick and easy methods of statistical analysis Transactions. *New York Academy of Sciences, Series II* 1953; 16: 88-97.
27. Anscombe FJ. Graphs in Statistical Analysis. *The American Statistician* 1973; 27: 17-21.
28. Devos SA, Van Der Valk PG. Epicutaneous patch testing. *Eur J Dermatol* 2002; 12: 506-13.
29. Rietschel RL, Fowler JF, Fisher AA. *Fisher's contact dermatitis*. Lippincott Williams and Wilkins, 2001.
30. Hicks S, Swindells KJ, Middelkamp-Hup MA, Sifakis MA, Gonzalez E, Gonzalez S. Confocal histopathology of irritant contact dermatitis in vivo and the impact of skin color (black versus white). *J Am Acad Dermatol* 2003; 48: 727-34.
31. Astner S, Burnett N, Ruiz-Diaz F, Doukas A, Gonzalez S, Gonzalez E. Irritant contact dermatitis induced by a common household irritant: A noninvasive evaluation of ethnic variability in skin response. *J Am Acad Dermatol* 2006; 54: 458-65.
32. Medenica M, Rostenberg Jr A. A comparative light and electron microscopic study of primary irritant contact dermatitis and allergic. *Contact Dermatitis* 1971; 56: 259-71.
33. Sinigaglia F, Scheidegger D, Garotta G, Scherper R, Lanzavecchia A. Isolation and characterization of Ni-specific clones from patients with Ni-contact dermatitis. *J Immunol* 1985; 135: 3929-32.
34. Kapsenberg ML, Res P, Bos JD, Schootemijer A, Teunissen MB, van Schooten W. Nickel-specific T lymphocyte clones derived from allergic nickel-contact dermatitis lesions in man: heterogeneity based on requirement of dendritic antigen-presenting cell subsets. *Eur J Immunol* 1987; 7: 861-5.
35. Garner LA. Contact dermatitis to metals. *Dermatol Ther* 2004; 17: 321-7.
36. Haberman SJ. 1978 [Analysis of qualitative data: Vol 1 Introductory topics, NY: Academic.111
37. de Fine Olivatus F, Menne T. Skin reactivity to metallic cobalt in patients with a positive patch test to cobalt chloride. *Contact Dermatitis* 1992; 27: 241-3.
38. Saglam AM, Baysal V, Ceylan AM. Nickel and cobalt hypersensitivity reaction before and after orthodontic therapy in children. *J Contemp Dent Pract* 2004; 15: 79-90.
39. Davis MD, Bhate K, Rohlinger AL, Farmer SA, Richardson DM, Weaver AL. Delayed patch test reading after 5 days: The Mayo Clinic experience. *J Am Acad Dermatol* 2008; 59: 225-33.
40. Larkin A, Rietschel RL. The utility of patch test using larger screening series of allergens. *Am J Contact Derm* 1998; 9: 142-5.
41. Saripalli YV, Achen F, Belsito DV. The detection of clinically relevant contact allergens using a standard screening tray of twenty-three allergens. *J Am Acad Dermatol* 2003; 49: 65-9.
42. Christensen GJ. Choosing an all-ceramic restorative material: porcelain-fused-to-metal or zirconia-based? *J Am Dent Assoc* 2007; 138: 662-5.
43. Holand W, Schweiger M, Watzke R, Peschke A, Kappert H. Ceramics as biomaterials for dental restoration. *Expert Rev Med Devices* 2008; 5: 729-45.