Effects of Base-Metal Casting Alloys on Cytoskeletal Filaments in Cultured Human Fibroblasts

Gülşen Can, DDS, PhD[a]
Gül Akpinar, DDS, PhD[b]
Alp Can, MD, PhD[c]

Purpose: The present study was designed to determine the cytotoxic effects of some widely used dental base-metal casting alloys (Ni-Cr and Co-Cr) on the cytoskeleton in cultured human fibroblasts, and to evaluate whether any structural alteration is associated with the application of these alloys. **Materials and Methods:** Ten specimens from six different alloys were prepared as 5-mm disks. Five of ten samples from each group were polished; the remaining five samples were left sandblasted with 50-μm Al_2O_3. All samples were directly exposed to human fibroblasts in a 24-well cell culture dish for 120 hours. Then, cells were fixed and stained with antibodies against major cytoskeletal elements—actin, vimentin, and microtubules—by immunofluorescent staining methods. Cells were analyzed in 3-D to document the cytoskeletal alterations using a laser confocal microscope. **Results:** Disintegration of actin filaments was observed in lamellipodia of fibroblasts by the effect of both polished and sandblasted Ni-Cr and Co-Cr samples, with the exception of the polished Co-Cr alloy (Wirocast). Moreover, intracytoplasmic actin-decorated stress fibers were found bent and occasionally tangled in the sandblasted Ni-Cr (Wiron 99) and Co-Cr alloys (Wirocast and Co-Cr Degussa). Vimentin, a mesenchymal cell intermediate filament protein normally showing an intracellular meshwork pattern, was not affected by any of the polished or sandblasted alloys. Microtubules mainly remained intact in all dental alloy–treated groups. **Conclusion:** Taken together, it is possible to postulate that Ni-Cr and Co-Cr dental alloys, especially sandblasted forms, may have detrimental effects on the actin-based cytoskeleton, at least tested in vitro. *Int J Prosthodont 2004;17:45–51.*

Dental casting alloys with high and low noble metal contents are widely used in dentistry. These alloys usually have multiphase structures and contain several metals that are known to be biologically active. The release of elements from these alloys occurs by corrosion and has been studied extensively.[1–3] Since the biocompatibility of Ni, Be, Cr, Co, and Mo is controversial, the present study aimed to test whether these base-metal alloys have any cytotoxic effect in the oral microenvironment. Intraoral release of elements is high at first, decreases with passivation, and remains constant in time. Obviously, the release of elements has to reach to a certain amount to cause harmful effects on the tissues. Studies have shown that the in vitro cytotoxicity of dental casting alloys may correlate with the release of elements from the alloys.[1–3]

Biocompatibility testing can be traditionally performed in three ways: in vitro tests (cell culture),[4–11] in vivo tests (applied in animals), and usage tests (tests performed in humans).[12] Cell culture tests have evaluated the cellular response to dental casting alloys. Cytotoxicity is generally assessed by measuring a certain type of cellular activity, such as morphology, viability, cellular proteins, hemolysis of human red blood cells, and membrane states.[12–25] Cell shape, motility, mechanical strength, and intracellular transportation mechanisms are carried out by a group of filamentous proteins in the cell, called the cytoskeleton. Therefore, any substance that is potentially toxic would have a negative effect on cytoskeletal organization of the cell.

[a]*Professor, Department of Prosthetic Dentistry, Ankara University Faculty of Dentistry, Besevler, Ankara, Turkey.*
[b]*Research Assistant, Department of Prosthetic Dentistry, Ankara University Faculty of Dentistry, Besevler, Ankara, Turkey.*
[c]*Professor, Department of Histology and Embryology, Ankara University Faculty of Medicine, Sihhiye, Ankara, Turkey.*

Correspondence to: *Prof Dr Alp Can, Ankara Universitesi Tip Fakültesi, Histoloji-Embriyoloji ABD, Sihhiye 06339 Ankara, Turkey. Fax: + 90 312 3106370. e-mail: alpcan@medicine.ankara.edu.tr*

Table 1 Base-Metal Alloys and Their Elemental Compositions (wt%)

Dental alloy	Co	Cr	Ni	Mo	Fe	Si	C	Ce	Nb
Ni-Cr									
Wiron 99, Bego	—	22.5	65.0	9.5	0.5	1.0	0.02	0.5	1.0
Wirocer, Bego	—	22.0	66.5	9.0	0.5	1.6	—	0.4	—
Duceranium U, Degussa	—	21.0	60.0	4.5	—	—	—	—	3.0
Co-Cr									
Wironit, Bego	64.0	28.7	—	5.0	—	1.0	0.40	1.0	—
Wirocast, Bego	33.0	30.0	—	5.0	29.0	1.0	0.30	0.3	0.2
Co-Cr Degussa, Degussa	63.0	28.0	1.0	5.0	—	1.0	1.00	1.0	1.0

The aim of the present work was to study the detrimental effects of the surface state of dental casting alloys on the main cytoskeletal fibers (ie, microtubules, actin microfilaments, and vimentin intermediate filaments) that predominantly determine the cellular morphology of cultured fibroblasts.

Materials and Methods

Preparation of Dental Alloys and Cell Cultures

Ten specimens from each of the six alloys tested (Table 1) were provided in the form of 5-mm-diameter disks, 2 mm in thickness with a 7-mm stem attached to one face to facilitate handling. Five of ten samples from each group were polished separately using a conventional technique to avoid cross-contamination. The remaining five samples were left sandblasted with 50-μm Al_2O_3. Each sample was then cleaned by a soft toothbrush, rinsed in distilled water, and ultrasonically treated in 99% ethanol for 15 minutes. After cleaning, the samples were placed in the center of glass coverslips 13 μm in diameter in 24-well cell culture dishes. Cells from a human embryonic cell line that was originally developed from an aborted human embryo (Ethical Committee No. 126-1993) as a primary fibroblast culture (isolated by one of the authors in 1993) were inoculated (10^4 cells/well) into these wells in a 37°C, humidified, 5% CO_2 atmosphere in 0.5 mL Dulbecco's Modified Eagle's Medium–Ham's F-12 cell culture medium (pH 7.4; Sigma) containing penicillin (100 IU/mL), streptomycin (100 mg/mL), amphotericin B (2.5 mg/mL), and sodium bicarbonate (1.2 g/L). Surface area of an alloy disk exposed to the cells was 0.195 cm^2, while the total surface area of cells plated was 1.130 cm^2.

The growth of cells was visually inspected by phase contrast microscope every 12 hours. The culture medium was changed every 72 hours. Cells were cultured in contact with the samples for 5 days. Cells cultured without alloy samples under the same conditions were used as a control group.

Fixation, Immunostaining, and Observation of Cultured Fibroblasts

After 120 hours of culture, the medium and the alloy disks were removed from the cell culture wells. Cells were washed with Dulbecco's phosphate-buffered saline (PBS) and fixed in a microtubule stabilization-extraction-fixation buffer (0.125 M Pipes, pH 6.9, 5 mM $MgCl_2 \cdot 6H_2O$, 2.5 mM EGTA containing 2.0% formaldehyde, 0.1% Triton X-100, 1 μM taxol, 0.01% aprotinin, 1 mM dithiothreitol, and 50% deuterium oxide)[26] for 10 minutes at 37°C and stored in PBS until staining procedures.

The microtubules were labeled with a 1:1 mixture of mouse monoclonal anti-alpha and anti-beta tubulin antibodies (1:100 in PBS; Sigma) at 37°C for 90 minutes in a dark and humidified chamber. Cells were washed three times in PBS and incubated in the same conditions with an FITC-conjugated sheep anti-mouse IgG antibody (1:100 in PBS; Jackson ImmunoResearch). Finally, cells were washed three times in PBS and mounted on slides with glycerol at 50% (v/v) in PBS.

Actin and vimentin filaments (Figs 1 to 3) were stained on the same slides as follows. Actin filaments were labeled with the rhodamine phalloidin (1:10 in PBS; Molecular Probes), a rhodamine-conjugated mushroom toxin that specifically binds to actin filaments. Vimentin intermediate filaments were labeled using mouse-monoclonal anti-vimentin antibody (1:200 in PBS; Sigma) at 37°C for 90 minutes in a dark and humidified chamber. Cells were washed three times in PBS and incubated in the same conditions with an FITC-conjugated sheep anti-mouse IgG antibody (1:100 in PBS; Jackson ImmunoResearch). Finally, cells were washed three times in PBS and mounted on slides with glycerol at 50% (v/v) in PBS.

Mounted coverslips were examined with a Zeiss LSM-510 Meta confocal microscope using 488-nm argon ion and 543-nm green He-Ne lasers at 40× and 63× plan-apo objectives. The confocal microscope enabled three-dimensional image acquisition by removing the out-of-focus images excited by two

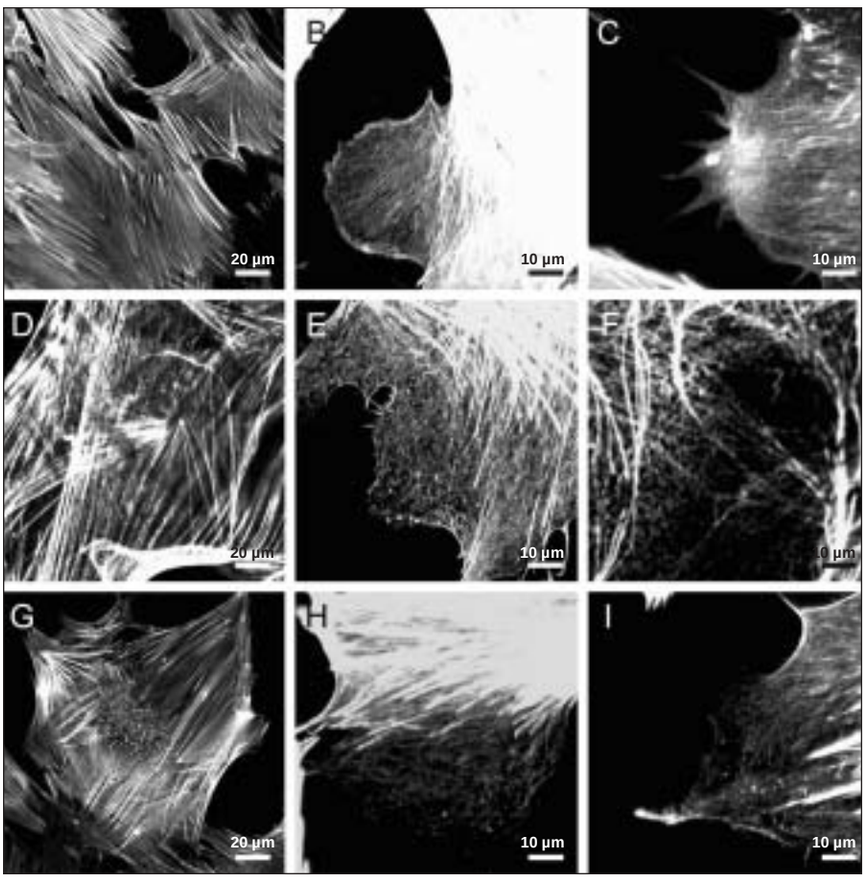

Fig 1 Fluorescent-labeled actin filaments in cultured human fibroblasts are seen in control samples as cytoplasmic stress bundles *(A)*, slender fibers in lamellipodia *(B)*, and in microspikes *(C)*. Note the delicate network of the actin filaments in lamellipodia and microspikes that extend from the cytoplasmic core to the lamellipodial ends forming the focal adhesion plaques. Treatment of cells with base-metal alloys did not demonstrate a gross change in stress fibers *(D* and *G)*. However, it caused a profound effect on the fine structure of those cell processes, as seen in the severe disintegration of actin filaments *(E, F, H,* and *I)*. *D* and *E* = polished Ni-Cr Wiron 99; *F* = sandblasted Ni-Cr Wiron 99; *G* and *H* = polished Co-Cr Degussa; *I* = sandblasted Co-Cr Degussa. Punctate staining of leftover actin filaments was located randomly within the lamellipodia.

monochromatic lasers, allowing recording of high-resolution digital images as optical sections.

Results

Actin Filaments

The actin-based intracytoplasmic filaments, called stress fibers, of a healthy cultured fibroblast were arranged as straight, long bundles throughout the cytoplasm (Fig 1a) and extended between the flattened extensions of cytoplasm called lamellipodia (Fig 1b), where tiny protrusions called microspikes were located at leading and lateral edges (Fig 1c). Lamellipodia and microspikes did not contain any organelles. Two major results were obtained in polished and sandblasted Ni-Cr– and Co-Cr–treated groups: *(1)* disintegration of lamellipodial actin filaments, and *(2)* bending of cytoplasmic stress fibers that dramatically altered the cell shape.

In most of the Ni-Cr and Co-Cr groups, actin filaments did not seem to demonstrate any perturbed structure in cytoplasmic stress fibers (Figs 1d and 1g). However, when cells were detected carefully in higher magnifications, disintegration of stress fibers was conspicuous in lamellipodia when compared to controls. All polished and sandblasted Ni-Cr and Co-Cr groups, except the polished Co-Cr Wirocast (not shown), displayed this phenomenon (Figs 1e, 1f, 1h, and 1i). Bending and curling of actin microfilaments were rarely observed compared to the disintegration of lamellipodial actin filaments. Only the sandblasted forms of the Ni-Cr alloy Wiron 99 (Fig 2b) and Co-Cr alloys Wirocast (Fig 2c) and Co-Cr Degussa (Fig 2d) caused the bending and curling of cytoplasmic stress fibers. The actin-based results summarized above were homogeneous in all cells, with no regard to the proximity of cells to the metal alloy disk in the cell culture well.

Vimentin Filaments

Vimentin-type intermediate filaments that normally show a meshwork throughout the entire cytoplasm were not affected by any of the polished or sandblasted base-metal alloys (Fig 3).

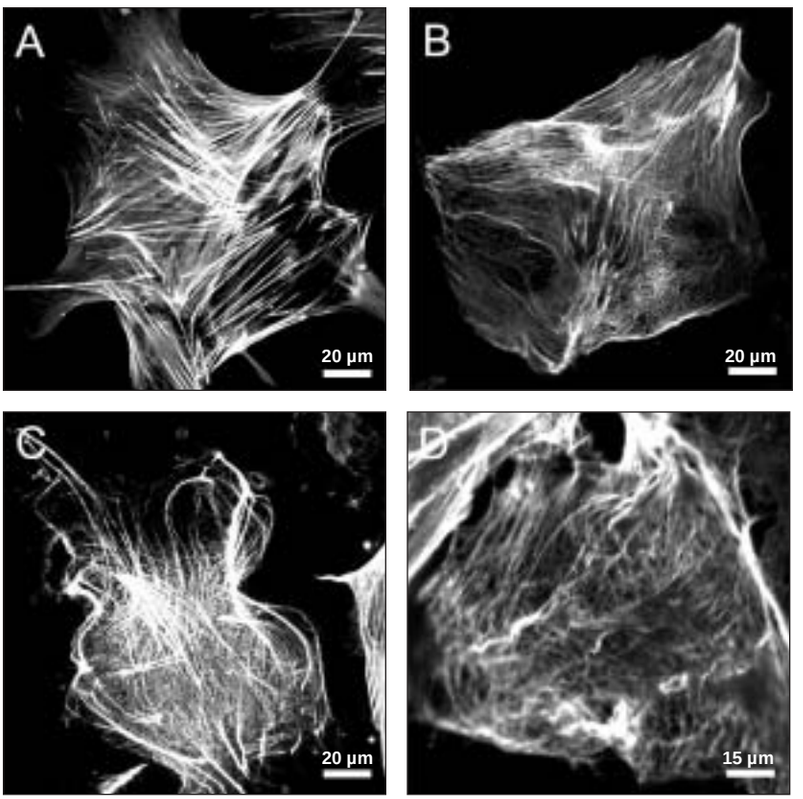

Fig 2 Bending and curling of actin-based stress fibers are observed in the control *(A)* and sandblasted Ni-Cr alloy Wiron 99 *(B)*, Co-Cr alloy Wirocast *(C)*, and Co-Cr Degussa *(D)*. Note the shrinkage or even disappearance of lamellipodia in these cells.

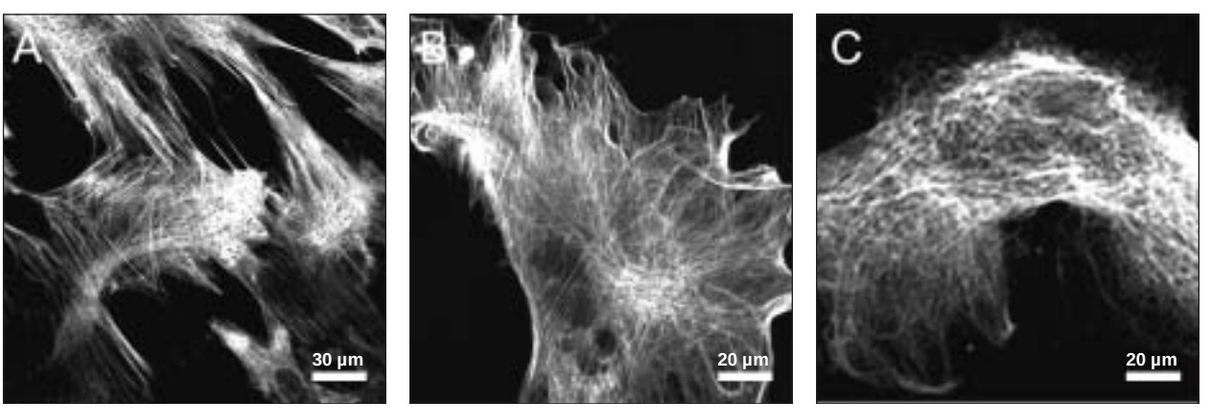

Fig 3 Vimentin does not show any sign of disturbance upon treatment with polished *(B)* or sandblasted *(C)* base-metal alloys when compared to controls *(A)*. Here, only polished Ni-Cr *(B)* and sandblasted Co-Cr *(C)* samples are presented. Unusual shape of the cell in *C* is possibly due to the impairment of actin filaments as shown in Fig 2.

Microtubules

Microtubules that tend to radiate from the cell center (centrosome) to the periphery were not basically affected by any of the polished or sandblasted alloys. Dual fluorescent labeling with antibodies raised against alpha- or beta-tubulin dimers did suggest that neither alpha- nor beta-tubulin polymers were affected (Fig 4).

Discussion

Addition of elements to metal alloys for providing various properties causes harmful effects to organisms.[1,2,16–25] Some elements might be harmful even in low concentrations.[12–25] Therefore, biocompatibility of a dental material is critical, and cellular effects are evaluated with cytotoxicity tests. Evaluation of biocompatibility in cell culture tests of dental

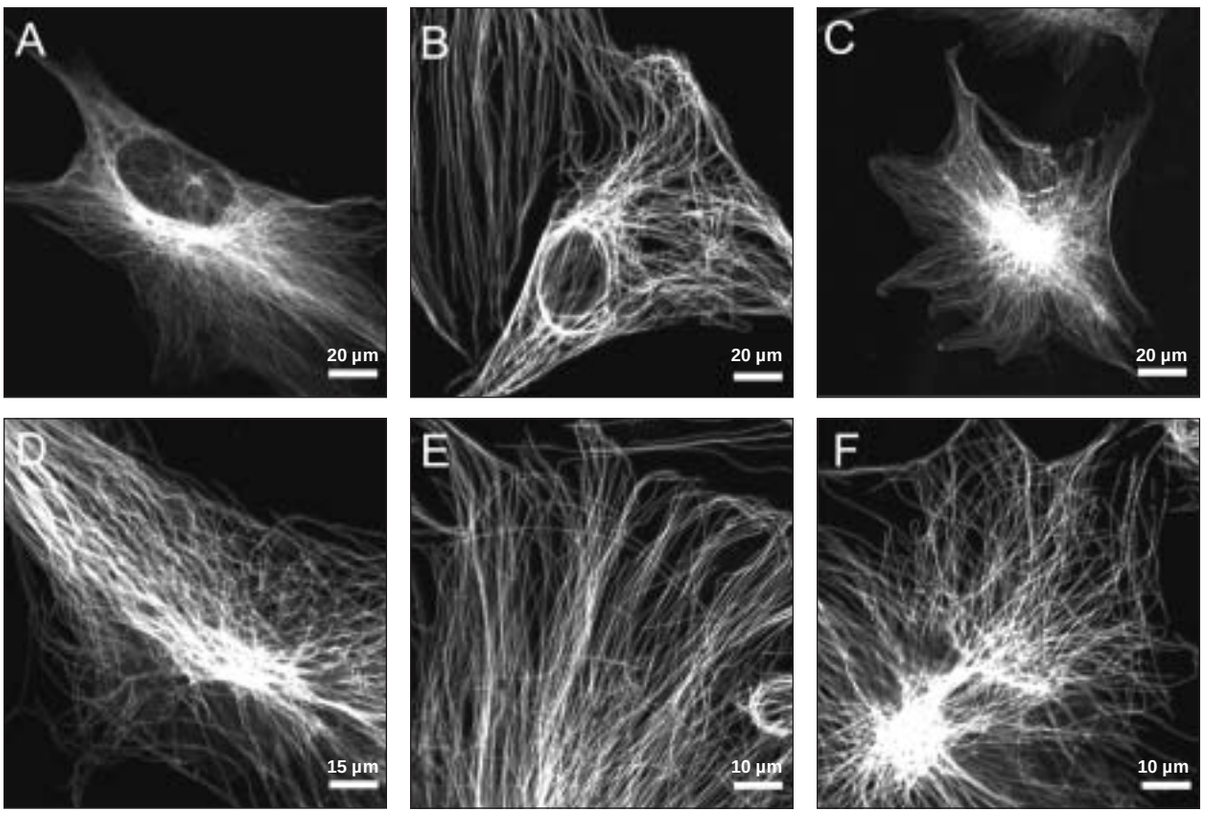

Fig 4 Microtubules labeled using tubulin antibody raised against alpha- and beta-tubulin dimers demonstrate a radiating fashion from the cell center throughout the cytoplasm. *A* = control; *B* = sandblasted Co-Cr Wironit; *C* = polished Ni-Cr Wiron 99; *D* = sandblasted Co-Cr Degussa; *E* = polished Co-Cr Wironit; *F* = polished Ni-Cr Duceranium U. No detectable destruction is noted in any of the samples presented.

casting alloys directly in contact with cells is well-accepted. Many studies have investigated the cytotoxicity of elements released from dental alloys.[6,12–25,27,28]

In the present study, the cytotoxicity of dental casting alloys was assessed regarding the morphology of actin microfilaments, intermediate filaments (vimentin), and microtubules. The base-metal casting alloys tested did not grossly alter the morphology of in vitro–exposed cells. However, mild to moderate degradation of actin-based filaments, particularly in growing tips of the cells, indicated onset of a destructive mechanism that might result in the complete loss of cells if the culture period extends. The degradation of these intracellular fibers that are responsible for promoting cell movement, maintaining cell shape, and organizing intracellular organelles will eventually have an effect on cell function. Briefly, microtubules and actin microfilaments are important in keeping the cell skeleton together, coordination of intracellular events, and material transport. Vimentin filaments comprise the major cytoskeletal element that stabilizes the cell shape and organelle localization in mesenchymal cells. The base structure of these filaments is a group of proteins of different molecular structure and behavior. For instance, while microtubules possess a dynamic instability property that enables them to polymerize/depolymerize within minutes or even seconds, vimentin and actin filaments are rather stable and more resistant to sudden changes. Therefore, the data obtained here will demonstrate a structural response of cells to dental alloys, for which relatively little knowledge has been accumulated in the literature.

Fibroblasts are major cellular components of the gingival connective tissue and are consequently affected by released elements of dental alloys. Macrophages and fibroblasts are much more sensitive to elements released from dental alloys.[12–25,27–30] Human gingival fibroblasts obtained from primary explant cultures were not preferred in this study because of their slow development and growth rate. Our preliminary experiments showed that this finite human

embryonic fibroblast cell line is the most appropriate tool for in vitro assays for three reasons: *(1)* they were cultivated from a human primary culture; *(2)* they grow much faster than adult fibroblasts, thus allowing follow-up of several rounds of cell division; and *(3)* they express vast amounts of cytoskeletal proteins simply detectable by immunofluorescent staining techniques. According to the literature,[5,7,11,15,25,29] 120 hours was chosen for the evaluation period. Craig and Hanks[4,5] tested the effect of cast and polished base-metal alloys on the morphology of culture fibroblasts. In contact with sandblasted Ni-Cr and Co-Cr, cellular morphology is profoundly affected compared to polished alloys.

Breakage of lamellipodial actin microfilaments was found in almost all cells. This has been thought to be related to an increase in the reversion of F-actin (fibrillar) to G-actin (globular) as a result of an impairment of the polymerization-depolymerization balance. In addition to actin breakage, bending of cytoplasmic actin filaments intimately in contact with sandblasted Ni-Cr alloy Wiron 99, sandblasted Co-Cr alloy Wirocast, or Co-Cr Degussa is thought to occur primarily because of element release. As a result, cells tend to dissociate from the substrate. Few cells were completely freed from the substrate. It is therefore possible that cytoskeletal alterations are the primary target for the released elements. Obviously, this does not rule out the possibility that another cellular function, such as signaling pathway or energy metabolism, is primarily impaired rather than synthesis and/or assembly of cytoskeletal filaments.

Breakage of actin filaments seen in polished and sandblasted Co-Cr alloy groups was not as common in Ni-Cr alloy groups, a phenomenon that may be related to the higher toxicity of the nickel found in Ni-Cr alloys. The detrimental effects of Ni-Cr alloys were higher than those of Co-Cr alloys. It would seem reasonable to conclude that in Ni-Cr alloys, the severity of actin-based cellular functions depends on the cytotoxic potential of nickel ions released by corrosion. The varying results obtained with Co-Cr alloys have been shown to be related to different percentages of cobalt released from the alloys.[2,5,15,30] Lack of any change in actin filaments with polished alloys can be due to lesser release compared to sandblasted samples; observing more reaction with sandblasted metal surfaces on actin filaments validates the importance of the polishing procedure. Interestingly, morphologic disturbances arising from the actin filaments appeared not only in the cells closely localized to the metal samples, but in the entire fibroblasts. This supports the idea that detrimental effects of implants occur as a result of metal ion release, not a mechanical effect.

High-resolution immunofluorescent images enabled us to observe minute changes in actin filaments as described above. However, more quantitative approaches would facilitate the evaluation of changes on actin-based cytoskeleton. Measurement of G-actin protein by protein electrophoresis or image analysis such as signal distribution graphs and area measurements will eventually indicate the severity of the cytoskeletal changes in future studies.

Vimentin was not affected by base-metal alloys. More than 99% of the intermediate filaments remain intact, whereas microfilaments and microtubules can be easily distorted when a cell is exposed to lysis[31]; it is not unusual to find the vimentin filaments intact, at least for the tested doses and duration.

Microtubules found in cells that were in contact with sandblasted and polished alloys did not demonstrate any prominent alteration compared to the controls. However, in only two samples, staining discontinuities along microtubules were detected (not shown) in cells contacting two polished Co-Cr alloys (Wironit and Co-Cr Degussa). Microtubules are comprised of alpha- and beta-tubulin dimers organized in a very tight fashion. Therefore, periodic lack of staining along the microtubules led us to assume that either alpha- or beta-tubulin dimers could have been affected. The resolution of both staining and observing techniques would not allow us to prove this hypothesis. However, it is possible to test this by dual staining of microtubules with alpha- and beta-tubulin antibodies. Since alpha- and beta-tubulin monomers have different posttransitional properties, they could be altered differently depending on the state they maintain. For instance, the acetylated form of alpha-tubulin is more stabilized and therefore resistant to depolymerizing agents.[26]

Under clinical conditions, a clear in vivo tolerance is present for the levels of elements releasing from dental alloys in the short run. These results at the cellular level with this relatively short-term in vitro study suggest that it is essential to continue study concerning these alloys.

Acknowledgments

This study was financially supported by projects DPT 97 K 120560 to AC for setting up a confocal microscopy unit, 98 K 120730 to GC and GA, and 2001 K 120240 from the Ankara University Biotechnology Institute.

References

1. Brune D. Metal release from dental biomaterials. Biomaterials 1986;7:163–175.
2. Geis-Gerstorfer JG, Sauer KH, Passler K. Ion release from Ni-Cr-Mo and Co-Cr-Mo casting alloys. Int J Prosthodont 1991;4:152–158.

3. Bumgardner JD, Lucas LC. Cellular response to metallic ions released from nickel-chromium dental alloys. J Dent Res 1995;74: 1521–1527.
4. Craig RG, Hanks CT. Reaction of fibroblasts to various dental casting alloys. J Oral Pathol 1988;17:341–347.
5. Craig RG, Hanks CT. Cytotoxicity of experimental casting alloys evaluated by cell culture tests. J Dent Res 1990;69:1539–1542.
6. Wataha JC. Biocompatibility of dental casting alloys: A review. J Prosthet Dent 2000;83:223–234.
7. Kawahara H, Yamagami A, Nakamura M. Biological testing of dental materials by means of tissue culture. Int Dent J 1968;18: 443–467.
8. Messer RLW, Lucas LC. Evaluations of metabolic activities as biocompatibility tools: A study of individual ions' effects on fibroblasts. Dent Mater 1999;15:1–6.
9. Messer RLW, Lucas LC. Cytotoxicity of nickel-chromium alloys: Bulk alloys compared to multiple ion salt solutions. Dent Mater 2000;16:207–212.
10. Mjör IA, Hensten-Pettersen A. The biological compatibility of alternative alloys. Int Dent J 1983;33:35–40.
11. Naji A, Harmand MF. Study of the effect of the surface state on the cytocompatibility of a Co-Cr alloy using human osteoblasts and fibroblasts. J Biomed Mater Res 1990;24:861–871.
12. Wataha JC. Cytotoxicity tests to screen the biological performance of dental casting alloys. Biocompatibility, allergies and resistance to corrosion: 8 years of research. Neuchâtel, Switzerland: Metalor, 1996:27–31.
13. Wataha JC, Lookwood PE. Release of elements from dental casting alloys into cell-culture medium over 10 months. Dent Mater 1998;14:158–163.
14. Wataha JC, Malcolm CT. Effect of alloy surface composition on release of elements from dental casting alloys. J Oral Rehabil 1996;23:583–589.
15. Wataha JC, Craig RG, Hanks CT. The release of elements of dental casting alloys into cell culture medium. J Dent Res 1991;70: 1014–1018.
16. Wataha JC, Craig RG, Hanks CT. Precision of and new methods for testing in vitro alloy cytotoxicity. Dent Mater 1992;8:65–71.
17. Wataha JC, Hanks CT, Craig RG. The in vitro effects of metal cations on eukaryotic cell metabolism. J Biomed Mater Res 1991; 25:1113–1149.
18. Wataha JC, Hanks CT, Craig RG. In vitro synergistic, antagonistic and duration of exposure effects of metal cations on eukaryotic cells. J Biomed Mater Res 1992;26:1297–1309.
19. Wataha JC, Hanks CT, Craig RG. The effect of cell monolayer density on the cytotoxicity of metal ions which are released from dental alloys. Dent Mater 1993;9:172–196.
20. Wataha JC, Hanks CT, Craig RG. Uptake of metal cations by fibroblasts in vitro. J Biomed Mater Res 1993;27:227–232.
21. Wataha JC, Hanks CT, Sun Z. Effect of cell line on in vitro metal ion cytotoxicity. Dent Mater 1994;10:156–161.
22. Wataha JC, Hanks CT, Sun Z. In vitro reaction of macrophages to metal ions from dental biomaterials. Dent Mater 1995;11:239–245.
23. Wataha JC, Lookwood PE, Nelson SK, Bouillaguet S. Long-term cytotoxicity of dental casting alloys. Int J Prosthodont 1999;12: 242–248.
24. Wataha JC, Lookwood PE, Nelson, SK , Rakich D. In vitro cytotoxicity of dental casting alloys over 8 months. J Oral Rehabil 1999;26:379–387.
25. Wataha JC, Malcolm CT, Hanks CT. Correlation between cytotoxicity and the elements released by dental casting alloys. Int J Prosthodont 1995;8:9–14.
26. Can A, Albertini DF. M-phase specific centrosome-microtubule alterations induced by the fungicide MBC in human granulosa cells. Mutat Res 1997;373:139–151.
27. Hanks CT, Wataha JC, Sun Z. In vitro models of biocompatibility: A review. Dent Mater 1996;12:186–193.
28. Nelson SK, Wataha JC, Cibirka RM, Lockwood PE. Accelerated toxicity testing of casting alloys and reduction of intraoral release of elements. J Prosthet Dent 1999;81:715–720.
29. Arvidson K, Cottler-Fox M, Hammarlund E, Friberg U. Cytotoxic effects of cobalt-chromium alloys on fibroblasts derived from human gingiva. Scand J Dent Res 1986;95:356–363.
30. Woody RD, Huget EF, Horton JE. Apparent cytotoxicity of base metal casting alloys. J Dent Res 1977;56:739–743.
31. Lodish H, Berk A, Zipursky SL, Matsudaira P, Baltimore D, Darnell J. Molecular Cell Biology, ed 4. New York: Freeman, 2000:836–843.

Literature Abstract

Micromechanics/structure relationships in the human mandible.

This research obtained the micromechanical properties of human mandibular cortical bone as a function of orientation from TMJ to TMJ. A mandible obtained from a deceased 66-year-old woman free of bone disease was used. The mandible was cut into 2-cm blocks and embedded in poly methylmethacrylate. Micromechanical properties were analyzed using the UH3 scanning acoustic microscope (SAM) in the burst mode for high resolution. The coordinates system was defined such that the inferior border of the mandible is positioned on the x-y plane. x is along the anterior-posterior direction, y is in the horizontal direction, and z is in the superior-inferior direction. The osteonal orientations were almost parallel to the x-axis and eventually branched into two directions toward the coronoid process and condylar head. The SAM revealed that almost the whole area of the mandibular body was transversely isotropic in the plane perpendicular to the x-axis. In the parallel and oblique directions, all data were transversely isotropic with respect to the x-axis. Data of the perpendicular osteons were transversely isotropic with respect to the z-axis.

Nomura T, Gold E, Powers MP, Shingaki S, Katz L. *Dent Mater* 2003;19:167–173. **References:** 19.
Reprints: Dr Tsutomo Nomura, Department of Tissue Regeneration and Reconstruction, Reconstructive Surgery for Oral and Maxillofacial Region, Niigata University, 2-5274 Gakkocho-Dori, Niigata City 951-8514, Japan. e-mail: t-nomura@dent.niigata-u.ac.jp—*Tee-Khin Neo, Singapore*