Effects of Metal Combinations on Cytotoxicity Evaluation Using a Dynamic Extraction Method

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The effects of metal combinations on cytotoxicity were examined following dynamic extraction by freely gyrating two spherical metals in a glass vessel. The cell viabilities of an Au alloy, a Ag-Pd-Au alloy and Ti were little affected by combinations among three metals. Cell viability ranged between 60 and 80% when precious alloys were in combination with Co-Cr or Ni-Cr alloys. Ti showed a clear difference in cell viability either in combination with Co-Cr or Ni-Cr alloys. The cell viability of the Ti/Co-Cr alloy combination was the same as that of precious alloys/Co-Cr or Ni-Cr alloy combinations. However, in an analogy with Co-Cr alloy/Ni-Cr alloy combination, the Ti/Ni-Cr alloy combination depressed the cell viability below 20%. This suggested that when new metals are to be used in combination with dissimilar metals, the cytotoxicity of the metals could be evaluated in extraction conditions using the mutual dynamic contact of dissimilar metals.

Key words: Cytotoxicity, Metal, Dynamic extraction

INTRODUCTION

Cytotoxicity of biomaterials in vitro has been achieved with either direct or indirect contact between cells and biomaterials\(^1\). As the extraction method, static extraction has been dominant, although it appears to less reflect the clinical situations of dental treatment with metallic materials that are used in stress-bearing positions. However, we tried dynamic extraction for cytotoxicity evaluation, reported the differences in cytotoxicity between static and dynamic extractions\(^2,3\), and obtained a better understanding of the metallic biomaterials currently in use.

Syrjanen et al.\(^4\) studied the effect of alloy combinations using cell culture to assess the biological significance of the galvanic effect and the toxicity of the alloy. In addition to the galvanic effect, dynamic contact between two metals removes protective surface layers and yields wear debris, which is followed by increased corrosion.

In the present study, we examined the effects of the same or dissimilar combinations of metals on cytotoxicity using a dynamic extraction where two spherical metals moved freely in a glass vessel.
MATERIALS AND METHODS

Materials and extraction method

Five metals for prosthodontic use were chosen as summarized in Table 1. Metals were cast in a sphere of ø 9.1 mm, abraded manually with an aqueous slurry of 240-1,000 grit silicon carbide (Fujimi Kenmazai Kogyo, Nagoya, Japan), then finely polished with 0.3 μm alumina (Marumoto, Tokyo, Japan). The specimens were washed ultrasonically in deionized water and rinsed with acetone. The cast specimens were produced according to the manufacturers' recommendations.

A fifty ml glass vessel containing two specimens was sterilized with an autoclave, then 40 ml of Eagle's medium (pH 7.4) with no serum was poured into the glass vessels, and fixed in a gyrotory incubator (G24, New Brunswick Scientific, N.J., USA). Dynamic extraction of freely moving specimens was carried out at 200 rpm for 14 days under aseptic conditions. Static extraction was also carried out under contact conditions for the two specimens. The extracts were filtrated through a 0.22 μm membrane filter (Millex-GV, Millipore, MA, USA). Nine extracts were obtained for each combination and were used in the cytotoxicity test.

Cytotoxicity evaluation by neutral red assay

L-929 mouse fibroblast cells were cultured with Eagle's minimum essential medium (pH 7.4, 37°C), supplemented with 10% fetal bovine serum and used for cytotoxicity evaluation. A 0.1 ml suspension of 2×10⁶ cells/ml was poured into each well of 96-multiplates and incubated at 37°C in CO₂ for 24 hr. This was followed by medium exchange with extracts or filtrates containing 10% fetal bovine serum, and incubation at 37°C for 72 hr. At the end of the culture time, cells cultured in the 96-multiplates were treated with neutral red (50 μg/ml in culture medium) for 3 hr under the culture conditions, washed with PBS(-) containing 1% formaldehyde and then with 0.2 ml of a mixture of 1% acetic acid-50% ethanol to extract the dye⁵. The optical density was measured at 540 nm and cell viability was expressed as a percentage of the control culture.

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<th>Table 1 Metals used in this study</th>
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<td>Classification of metals</td>
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<td>Au based alloy</td>
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<td>Ag based alloy</td>
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<td>Ni based alloy</td>
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⁴Casting Gold M.C. Type, GC Co., Tokyo, Japan
⁵Castwell MC, GC Co., Tokyo, Japan
⁶cpTi JIS2, Toshinyokou Co., Nigata, Japan
⁷Vitallium, Austenal Co., Ill. USA
⁸Suncolium U, Sankin Co., Tokyo, Japan
Statistical Analysis
Values were expressed as the mean and standard deviation, and were statistically analyzed using a one-way ANOVA to compare the combinations, as the Levene test for homogeneity of variances showed that the variances were not different among the groups. An LSD multi-range test with a significant level of 0.05 was used to determine which combination means were significantly different from each other. A paired t test was used for the comparison between extracts and filtrates of the same combination.

RESULTS
Figs. 1 and 2 represent the cell viability of extracts obtained under static conditions. All combinations were the same as that of the control.

Cell viabilities of the dynamic extracts obtained from the same metal combinations are shown in Fig. 3. Cell viabilities of the Ag-Pd-Au alloy/Ag-Pd-Au alloy and Ti/Ti combinations were the same as that of the control. However, the mean cell viability decreased to 75.5% for the Au alloy/Au alloy combination, followed by 35.9% for the Co-Cr alloy/Co-Cr alloy combination, and 9.2% for the Ni-Cr alloy/Ni-Cr alloy combination, respectively. After filtration of the extracts as shown in Fig. 4, the cell viability was the same for those extracts, except for the Au alloy/Au alloy combination. In the case of the Au alloy/Au alloy combination, filtration caused a significant increase in cell viability (p<0.05).

Fig. 5 shows the cell viability of extracts obtained by dissimilar metal combinations. The Au alloy/Ti and Ag-Pd-Au alloy/Ti combinations showed a mean cell viability of greater than 90%. The mean cell viability of the Au alloy/Ag-Pd-Au alloy combination was 85.2% and was lower than that of the Au alloy/Ti and the Ag-Pd-
Au alloy/Ti combinations, but no significant difference was found among the three combinations. The mean cell viability of the Au alloy/Co-Cr alloy, Au alloy/Ni-Cr alloy, Ag-Pd-Au alloy/Co-Cr alloy, Ag-Pd-Au alloy/Ni-Cr alloy and Ti/Co-Cr alloy combinations varied between 61.4% for the Au alloy/Ni-Cr alloy combination and 73.6% for the Ti/Co-Cr alloy combination. Extracts of the Ti/Ni-Cr alloy and Co-Cr alloy/Ni-Cr alloy combinations caused a significant reduction in cell viability and showed no significant difference in cell viability compared with that of the Ni-Cr alloy/Ni-Cr alloy combination. In fact, the cell viabilities of the precious alloys/Ti combinations were significantly higher than those of the precious alloys/Ni-Cr or Co-Cr alloys combinations. The Ti/Co-Cr alloy combination, compared with the Ti/Ni-
Cr alloy or Co-Cr alloy/Ni-Cr alloy combinations, induced significantly higher cell viability. Filtration tended to increase the cell viability as shown in Fig. 6. However, only the Au alloy/Ag-Pd-Au alloy combinations revealed a discrepancy in their effects on cell viability between extracts and filtrates (P<0.05).

DISCUSSION

The present findings demonstrated that cell viability under dynamic extraction was influenced by a variety of metal combinations, and that there were differences in cell viability between extracts and filtrates in one or more combinations, when extraction was done with two spherical metals in dynamic contact with each other. Furthermore, under dynamic contact conditions, the cell viability differed considerably compared with static contact conditions, which showed no difference in cell viability among the combinations.

In the combinations with the same metals, the cell viability of the Au alloy/Au alloy combination decreased to 75.5%, while that of the Ag-Pd-Au alloy/Ag-Pd-Au alloy combination was 97.8%. It was reported that copper selectively dissolves from both alloys. However, the present findings indicated that the difference in wear debris and copper dissolution may have contributed to the difference in the cell viability of both alloys. Plenk reported that dental gold alloy debris significantly inhibited the growth of fibroblasts. It is, therefore, necessary to explain the discrepancy in cytotoxic findings among non-precious metals. The Ti/Ti combination was not toxic, but cell viability was depressed with the Co-Cr alloy/Co-Cr alloy or Ni-Cr alloy/Ni-Cr alloy combinations. Cell viability is related to the amount of dissolution from metals and the cytotoxic effect of dissolved metal ions. Titanium has a corrosion resistance superior to that of cobalt-chromium or nickel-chromium alloys. Furthermore, the IC50 (the concentration needed to depress cellular activity by 50%) of Ti ions is higher than that of Co or Ni ions. In addition, it was assumed that Ti dissolution and production of wear debris in the Ti/Ti combination were not sufficient to lead to a decrease in cell viability under the present experimental conditions. This may be because specimen preparations were made with the same surface areas among the combinations, which naturally led to a decrease in specimen weights because of the different specific gravities of each metal. The corrosion resistance of cobalt-chromium and nickel-chromium alloys increases with their chromium content. The Co-Cr alloy used in this study contained over 30% chromium and had good corrosion resistance. However, the present findings indicated that the Co-Cr alloy/Co-Cr alloy combination decreased cell viability, and that there was no difference in cell viability between extracts and filtrates. It is conceivable that Co or Cr ions dissolved from the Co-Cr alloy affected cell viability. According to Brune et al., a cobalt dissolution from a nonpassivated cobalt-chromium alloy was higher than that from a prepassivated cobalt-chromium alloy. This suggests that dissolution increases if the protective surface layer of cobalt-chromium alloy is broken. The above process is always followed by the production of wear debris. The cell viability
of the Ni-Cr alloy/Ni-Cr alloy combination would thus be depressed by nickel dissolution. Espevik\(^{12}\) reported that significant amounts of nickel and chromium were observed when the chromium content of the alloy was below approximately 16%.

The combinations of dissimilar alloys were divided into four groups according to cell viability. First, the Au or Ag-Pd-Au alloys/Ti combinations were nontoxic. Second, the Au alloy/Ag-Pd-Au alloy combinations showed a slight cytotoxic effect with the extracts, but no toxic effect with the filtrates. Third, the Au or Ag-Pd-Au alloys/Co-Cr or Ni-Cr alloys and the Ti/Co-Cr alloy combinations showed a mild cytotoxic effect. Finally, the Ti/Ni-Cr and Co-Cr alloy/Ni-Cr alloy combinations showed a severe cytotoxic reaction. These findings appeared to show that the cell viability of the combinations among the Au alloy, Ag-Pd-Au alloy and that of Ti would likely be influenced by production of wear debris from Au alloys, rather than metal dissolution. It is thought that the Au alloy did not wear in Au alloy/Ti combinations because Ti was lighter than Au or Ag-Pd-Au alloys. Actually, some golden deposits were observed on the filter of the Au alloy/Au or Ag-Pd-Au alloy combination, but were not observed on the filter of the Au alloy/Ti combination. In contrast, the third group appeared to mainly depend on metal dissolution from the Co-Cr or Ni-Cr alloys. However, it appears unlikely that metal dissolution was accelerated by dynamic contact of the Co-Cr or Ni-Cr alloys with the precious alloys. Gjerde\(^{13}\) also reported that the gold alloy exhibited low corrosion currents in contact with the cobalt-chromium alloy. However, Ti showed a difference in cell viability according to whether the combinations were Co-Cr or Ni-Cr alloys. Electrochemical tests indicated that titanium appeared to be compatible with cobalt-chromium alloy\(^{14}\). Although, nickel-chromium alloy in combination with titanium had a clearly differentiated corrosion behavior depending on the composition of the alloys\(^{15}\). The Ni-Cr alloy used in the present study may be susceptible to galvanic corrosion in combination with titanium due to its low chromium content. Consequently, the cell viability of the Ti/Ni-Cr alloy combination was lower than that of the Ti/Co-Cr alloy combination or the precious alloys/Ni-Cr alloy combination.

A difference in cell viability between extracts and filtrates was observed in the Au alloy/Au alloy, Au alloy/Ag-Pd-Au alloy, Au alloy/Co-Cr alloy and Au alloy/Ni-Cr alloy combinations. Wear debris, rather than metal dissolution, may be responsible for the injurious cellular effects in the Au alloy/Au or Ag-Pd-Au alloy combinations, as the cell viability recovered after filtration. Even if metallic biomaterials are not toxic due to excellent corrosion resistance, cytotoxic effects can occur when wear debris is produced by the action of mechanical forces. Consequently, such effects should be emphasized in cytotoxicity tests on metallic biomaterials, which are used under stress-bearing conditions, i.e. in the oral cavity. In such circumstances, impairment of the protective film and production of wear debris would recur, together with a galvanic effect. It is apparent that conventional static extraction would not be adequate for clinical situations of dental treatment with metallic materials used in stress-bearing positions which are sometimes in contact with the same or dissimilar alloys in the oral cavity. The present findings under
static conditions showed that no combinations had a significant effect on cell viability (Figs.1 and 2).

In summary, the present findings indicated that dynamic contact between two metals had an influence on cellular response, not only via a galvanic effect, but also by accelerated dissolution and production of wear debris. Friction between specimens and/or the inner side of a glass vessel must be considered in this extraction method. Differences in specimen weights were also a factor in the results, as discussed earlier. These factors may affect metal dissolution and production of wear debris, as well as cell viability. Further development of the dynamic extraction method will be necessary to allow quantitative comparisons. Nevertheless, understanding this process may lead to better selection of appropriate dental materials in clinical situations.

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REFERENCES


