Abstract: The aim of this research was to evaluate the effect of individual metallic elements within experimental Au-Pt-based metal-ceramic alloys on in vitro biocompatibility. A binary Au-10 at.% Pt alloy (AP10) was designed as a parent alloy. Six ternary AP10-X (X = In/Fe/Sn/Zn) alloys and four quaternary (AP10-In2)-Y (Y = Fe/Sn/Zn) with different compositions were cast into square plates with size 10X10X0.5 mm³ and subjected to porcelain-firing thermal cycling. A commercial alloy was used as a control. In vitro biocompatibility was investigated using L929 murine aneuploid fibrosarcoma cell line. The test samples and cells were incubated at 37°C in a 5% CO₂ atmosphere for 72 h. Alamar™ Blue Assay was carried out to determine the respiratory viability of cultures maintained in the presence of the different materials. The cell only control showed significantly higher levels of cell viability than all six of the ternary alloys and two of the four quaternary alloys, (AP10-In2)-Zn2.1 and (AP10-In2)-Sn1.0 (P < 0.05). The quaternary alloys showed slightly higher levels of cell viability than the ternary alloys, with the exception of AP10-Sn0.9. No statistical differences were seen between the ternary and quaternary alloy groups. Acceptable cell viability was observed on the surfaces of all the alloys. (J Oral Sci 53, 387-391, 2011)

Keywords: metal-ceramic alloys; in vitro biocompatibility; cellular activity.

Introduction

Porcelain-fused-to-metal (PFM) restorations are widely used in dentistry because of their excellent clinical performance. A widely accepted definition of biocompatibility is “the ability of a material to elicit an appropriate response in a given application”. This implies that there is an interaction between a host, a material and an expected function of the material. If these three factors are in harmony then a material can be said to be biocompatible (1).

At high enough levels metal ions can disable cellular metabolism (2) and decrease cellular proliferation (3). Metal ions released from dental alloys interact with metabolic pathways and cell structures causing damage (4). Extreme cases can see metal ions enter the circulatory system and be distributed systemically by proteins such as albumin. These ions may then induce gene activation in endothelial cells. Cation release can provide inflammatory reactions and may modulate the immune response by activation or inhibition of T- and B- cells (5). These responses can be in the form of oral mucositis, gingivitis/periodontitis and alveolar bone resorption (4).

The UK adverse reactions reporting project (6) showed that reactions to precious metals accounted for about 5% of the reactions caused by metals and the number of allergic causes attributed to metals appears to be small. Another study (7) found that in not more than 10% of patients was allergy diagnosed as contributing to

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a complaint or symptom. However, metal components from almost all cast dental alloys can be detected in adjacent tissue (8).

Phase formation plays a considerable role in determining the biocompatibility of dental alloys, with multi-phase Ag-Pd-Cu alloys showing more cytotoxicity than single-phase materials (9). When placing dental restorations adjacent to the gingiva and periodontium, non-precious alloys were found to almost completely inhibit cell viability while noble alloys and non-alloyed titanium showed better results (10).

Most studies have observed that the lower noble content alloys (those containing more base elements) produced stronger tissue reactions than the higher noble content and gold alloys (4,10,11). The oxide-forming elements (In, Fe, Sn, Zn) incorporated in the precious alloys for PFM restorations are base metal elements and generally tend to be more soluble compared to the noble metal elements. It has been reported that the extended exposures to low doses of metal ions may also have biological liabilities (12).

With the PFM restorations the majority of the metallic frame is covered with veneering porcelain. However, it is usual for its small collar to be left uncovered. This part is usually highly polished and partially sub-gingival. Since cell culture systems have been found to be suitable in-vitro models for testing the biocompatibility of dental alloys (13-15), it is important to know cell viability on the surface of the experimental Au-Pt-based PFM alloys containing small amounts of oxide-forming elements. The aim of this research was to study the effect of the individual oxide-forming elements within experimental Au-Pt-based PFM alloys on in vitro biocompatibility.

**Materials and Methods**

**Specimen preparation**

A binary Au-10 at.% Pt alloy (referred to as AP10) was designed as a parent alloy. Six ternary AP10-X (X = In/Fe/Sn/Zn) alloys and four quaternary (AP10-In2)-Y (Y = Fe/Sn/Zn) alloys with different compositions were designed on the atomic percentage basis and the amount of oxide-forming elements X and Y were restricted up to 2 at.%.

All the experimental alloys were prepared from high-purity component metals. Appropriate amounts of component pure metals were melted in a high-frequency induction furnace and the ingots obtained were subjected to cold rolling and homogenizing heat-treatments at high temperatures. A number of plate samples with size 10×10×0.5 mm³ were obtained. The analyzed composition in atomic percentage of the alloys used in the study can be seen in Table 1. Since the concentration of oxide-forming elements, In/Fe/Sn/Zn, was restricted up to 2 at.% in the present experimental alloys, these elements were mostly dissolved in the parent Au-Pt phase and no distinct sign of the presence of the second phase was recognized. A commercially produced Au-Pt-based alloy BiOcclus 4® (DeguDent GmbH, Postfach 1364, D-63403 Hanau, Germany) was investigated also for comparison.

All eleven alloys were then lost wax cast into square plates 10×10×0.5 mm³ and ground smooth to simulate the preparation of a metal-ceramic coping. The plates were then placed into a dental porcelain furnace and subjected to oxidising, core, main and glazing firing cycles (Oxidation 980°C in air for 5 min, Core 950°C in vacuum for 3 min, Main 930°C in vacuum for 6 min and Glaze 930°C in air for 1 min) to simulate what the alloys would have been subjected to during the manufacture of a porcelain-fused-to-metal (PFM) restoration.

All the square plates were then highly polished to a clinically acceptable state on both their square faces and all sides using fine stones (Meisinger, Germany), rubber wheels (Identoflex AG, Buchs SG, Switzerland) and bristle brushes and fine lamb’s wool mops (C&LE Attenuborough Ltd, Nottingham, UK) loaded with universal polish (yellow and green polish for precious metals, Metrodent, Huddersfield, UK) to remove the oxide layer from the surface of the alloy and to replicate the polishing that would be undertaken on the exposed alloy palatal/lingual gingival collars of finished restorations.

**In vitro biocompatibility**

Alloy samples (n = 4) were sterilised by autoclaving (15 min at 121°C) prior to use, this had no visual effect on the polished surfaces of the alloys. Tissue culture plastic was used as a control. In vitro biocompatibility was investigated using L929 murine aneuploid fibrosarcoma cell line (American Type Culture Collection, ATCC; Rockville, MD). The cells were seeded (density of 1.25 × 10⁴ cells.ml⁻¹) into wells of a well plate containing one piece of each alloy being tested with a total well volume of 1 ml. This was repeated a further three times in separate well plates. The materials and cells were incubated at 37°C in a 5% CO₂ atmosphere for 72 h. Alamar™ Blue Assay was carried out to determine the metabolic activity of cultures maintained in the presence of the different test materials using the manufacturer’s standard protocol. The cell viability was expressed as a percentage relative to the material-free control. Scanning electron microscopy (SEM) was used to determine the morphology of the cells grown on the materials surface. These methods
have been reported previously for the evaluation of in vitro biocompatibility (16).

Statistical analysis

The results were analysed using two-way analysis of variance (ANOVA) at the 95% confidence level ($P = 0.05$). The Newmans-Kuel multiple comparison summary was used to indicate significant differences. Individual comparisons were analysed by using a paired $t$ test. Normal distribution tests were also carried out to determine whether the data obtained was significantly different from that, which would normally expected from this data (i.e. was the data probably normal).

Results

Figure 1 shows the percentage relative cellular activity (%) of cells cultured in the presence of the experimental alloys tested. The cell only control showed significantly greater cellular activity than the ternary alloys: AP10-In1.7, AP10-Fe1.9, AP10-Fe0.8, AP10-Zn1.7, AP10-In1.0 and AP10-Sn0.9 ($P < 0.05$).

The cell only control also showed a significantly higher level of cell activity when compared to two of the quaternary alloys, (AP10-In2)-Sn1.0 and (AP10-In2)-
Zn2.1 ($P < 0.05$). However, two of the quaternary alloys, (AP10-In2)-Fe1.7 and (AP10-In2)-Fe1.0 did not show any significant difference when compared to the cell only control ($P > 0.05$).

No significant differences were noted between the cellular activity recorded in the presence of the 6 ternary or on the surfaces of the 4 quaternary alloys. No significant differences in cellular activity were observed between the ternary and quaternary alloy groups ($P < 0.05$).

The commercial alloy, BiOcclus 4®, showed greater cellular activity than the ternary alloys ($P > 0.05$), with the exception of the AP10-Sn0.9 and AP10-In1.0 alloys, but did not show any significant differences compared to the quaternary alloys ($P > 0.05$).

Normal distribution analysis of the cell activity on all 4 samples of each of the eleven alloys tested in the study and the cell only control showed that there were no significant differences from those normally expected ($P > 0.05$). This confirmed that the data collected fell within the normally expected distribution range.

Scanning electron microscopy confirmed the quantitative biocompatibility results. Figures 2a and b illustrates the difference in cellular activity observed on the surface of the test alloys. Figure 2a shows an even distribution of fibroblast-like cells on the surface of the quaternary alloy (AP10-In2)-Zn2.1. Figure 2b shows a SEM micrograph of L929 cells cultured on the surface of the ternary alloy AP10-In1.7 and shows a poor cellular response to the test alloy, very few cells are noted on the surface and their morphology is rounded.

### Discussion

With the ternary alloys the AP10-Sn0.9 alloy showed the most cellular activity with the AP10-In1.0 and AP10-Zn1.7 alloys and Fe-containing AP10-Fe0.8 and AP10-Fe1.9 alloys having slightly less cellular activity, with only the AP10-In1.7 alloy showing decreased cellular activity (Fig. 1). We previously investigated ion release from the same series of the Au-Pt-based alloys into the deionized water and found that the order of the amount of dissolved metallic ions was Fe > Zn > In > Sn (17). Furthermore, Fe showed significantly higher levels of ion release than the other base metal elements (Zn, In, Sn) (17). The results of the previous ion release tests well explain the present experimental results of the highest cellular activity of the AP10-Sn0.9 alloy. Relatively lower cellular activity observed on the Fe-containing ternary alloys AP10-Fe0.8 and AP10-Fe1.9 may be related to significantly higher levels of released Fe ions (17). Considering the facts that the absolute amounts of released In ion from In-containing ternary alloys into deionized water were small and that slightly increased amount of In ion was released from the AP10-In1.7 alloy than from the AP10-In1.0 alloy (17), the present cellular viability results show that the cellular viability on the AP10-In1.7 alloy was slightly lower than that on the AP10-In1.0 alloy and this may explain the strong adverse effect of In ion on cell viability.

The observations from this work suggest that quaternary alloys showed slightly improved cellular activity than the ternary alloys. This may be considered unusual as when we look at the quaternary alloys they contain Au, Pt and In with the additions of Fe, Sn and Zn, however, when we look at the results for the alloys which contain just Au, Pt and In (AP10-In1.7 and AP10-In1.0) we see that they showed less cellular activity than all the quaternary alloys. This would seem to suggest that cells prefer to have Au, Pt and In plus other elements. For the quaternary alloys Fe showed the greatest cellular activity.

### Table 1 The chemical composition in at.% of the 11 Au-Pt-based noble alloys used in the study

<table>
<thead>
<tr>
<th>Alloys</th>
<th>Au</th>
<th>Pt</th>
<th>In</th>
<th>Fe</th>
<th>Zn</th>
<th>Sn</th>
<th>Other Rh &amp; Ta</th>
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<tbody>
<tr>
<td>AP10-In1.0</td>
<td>89.1</td>
<td>9.9</td>
<td>1.0</td>
<td>0</td>
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<td>AP10-In1.7</td>
<td>88.4</td>
<td>9.9</td>
<td>1.7</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AP10-Fe0.8</td>
<td>89.2</td>
<td>10.0</td>
<td>0</td>
<td>0.8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AP10-Fe1.9</td>
<td>88.3</td>
<td>9.8</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AP10-Zn1.7</td>
<td>88.5</td>
<td>9.8</td>
<td>0</td>
<td>1.7</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>AP10-Sn0.9</td>
<td>89.2</td>
<td>9.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0.9</td>
<td>0</td>
</tr>
<tr>
<td>(AP10-In2)-Fe1.0</td>
<td>87.3</td>
<td>9.7</td>
<td>2.0</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(AP10-In2)-Fe1.7</td>
<td>86.6</td>
<td>9.7</td>
<td>2.0</td>
<td>1.7</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>(AP10-In2)-Zn2.1</td>
<td>86.3</td>
<td>9.6</td>
<td>2.0</td>
<td>0</td>
<td>2.1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>(AP10-In2)-Sn1.0</td>
<td>87.3</td>
<td>9.8</td>
<td>1.9</td>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>0</td>
</tr>
<tr>
<td>BiOcclus 4</td>
<td>83.3</td>
<td>10.8</td>
<td>2.8</td>
<td>0</td>
<td>1.5</td>
<td>0</td>
<td>1.6</td>
</tr>
</tbody>
</table>
closely followed by Zn and Sn, though the standard deviation for the (AP10-In2)-Fe1.7 alloy was the highest of any alloy tested.

The commercial alloy, BiOcclus 4®, showed very good in vitro biocompatibility and its composition is very similar to the quaternary alloy (AP10-In2)-Zn2.1 (Table 1) with the exception of Rh and Ta and although not statistically different from this alloy, did exhibit greater cellular activity.

All the alloys and elements tested in the study showed an ability to have cells grow on them, which is very encouraging given the popularity that noble-metal alloys still have as restorative materials. Being able to determine the relative cellular activity of alloys that are likely to come into contact with the oral tissues will help enormously with alloy selection for certain clinical situations.

The effects of individual metallic elements within experimental Au-Pt-based metal-ceramic alloys were examined by in vitro cell viability tests and the following conclusions were drawn:

- The cell only control showed significantly more cell viability than all the ternary alloys and two of the quaternary alloys, (AP10-In2)-Sn1.0 and (AP10-In2)-Zn2.1.
- The quaternary alloys in the study showed slightly more cell viability than the ternary alloys, with the exception of the AP10-Sn0.9 alloy but no statistical differences were seen between the two groups.
- It would appear that a mix of In and other oxide-forming elements (Fe, Sn, Zn) creates a surface, which is conducive to cell viability.
- BiOcclus 4® and the quaternary alloys perform better than the ternary alloys.
- All the alloys/elements tested showed clinically acceptable levels of cell viability.

References