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# Evaluation of Tissue Reaction to Some Denture-base Materials: An Animal Study

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## Abstract

**Aim:** Controversy continues regarding the biocompatibility of denture base materials. One method to evaluate the biocompatibility of materials is in an animal study. Using dogs as subjects, the purpose of this study was to evaluate the vestibular tissue reaction to cobalt chromium (Co-Cr), heat cure acrylic resin, and acrylic resin mixed with aluminum oxide ($Al_2O_3$) compared with a control group using the histopathologic method.

**Methods and Materials:** Twelve disk shape samples (2 mm x 8 mm) in four groups of Co-Cr, acrylic resin, acrylic resin mixed with a 20% weight ratio of $Al_2O_3$, and a control group (Teflon) were fabricated. In one stage surgery two samples of each material (8 samples) was implanted in the buccal vestibule of each dog (n=6), subcutaneously. At 45 and 90-day intervals, half of the samples were excised along with peripheral tissue to assess the presence of inflammation by grading on a scale from 0 to 3 and the presence of a fibrotic capsule using histological observations. Data were analyzed using the Kruskal-Wallis, Mann-Whitney, and Tau b Kendal tests.

**Results:** Tissue reaction between Co-Cr and the control group was significant (P=0.02), but it was not significant between other groups. There was no significant difference between the 45 and 90-day post-insertion samples. The formation of fibrotic capsule groups was significant (P=0.01). It was significant between the Co-Cr and acrylic resin groups (P=0.01) and the acrylic resin and control groups (P=0.01).

**Conclusion:** The Co-Cr group was more toxic than the other groups. The inflammation increased during time. The inflammation in two acrylic groups was greater than the control and less than the Co-Cr group. The formation of fibrotic capsule, except in the acrylic resin with $Al_2O_3$ group, increased over time.

**Clinical Significance:** Co-Cr alloys are toxic and can produce damage to living tissue. Heat cure acrylic resin materials have less toxicity, and their use is safer than Co-Cr alloys.

**Keywords:** Heat cured acrylic resin, cobalt chromium, Co-Cr, aluminum oxide, $Al_2O_3$, tissue reaction

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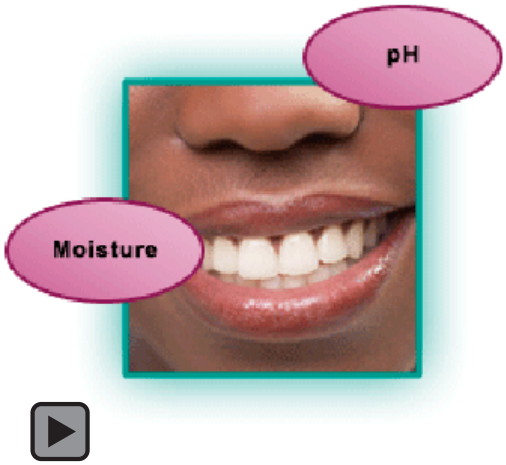
## Introduction

Biocompatibility and cytotoxicity of denture base materials which are in contact with oral mucosa are still contradictory.[1] When used, these materials are in direct contact with oral tissues for a prolonged period of time; the biologic factors unique to the oral environment such as pH and thermal changes, moisture, microorganisms and enzymes can change the chemical and physical properties of these materials.[2]




Denture-base alloys usually have a multiphase structure containing several metals. Corrosion causes the release of elements from these alloys that may have a cytoxic effect in the oral environment.[3] On the other hand, the biologic characteristics of denture-base resins are influenced by the monomer to polymer conversion. Despite the various methods used to initiate the polymerization of acrylic resin, the conversion of monomers to polymers is incomplete leaving residual monomer in the denture base that can leach into the saliva. The unreacted monomer can cause adverse reactions in the oral mucosa in contact with the denture.[4] On the other hand, acrylic resin has a low thermal conductivity compared with denture-base metal alloys. Studies have shown thermal diffusivity to be an important material property in determining the gustatory response. Other studies experimented with increasing the thermal conductivity of acrylic resin by adding metal[5] or ceramic filler.[6,7]

Adding ceramic filler can improve some physical properties of acrylic resins.[8] Therefore, a study of the biologic properties of a mixture of acrylic resin and ceramic filler is essential. Biocompatibility testing can be conducted using *in vitro* tests (using cell cultures), *in vivo* tests (animal studies), or clinical trials (human testing).[9] The aim of the present work was to study the biologic reaction of cobalt chromium (Co-Cr) alloy, a heat-cure acrylic resin denture-base material, and a mixture of heat-cure acrylic resin and ceramic filler (aluminum oxide) ($Al_2O_3$) which was implanted in the vestibular mucosa of dog mouths using the histopathologic method.

## Methods and Materials

### Subjects

Six Iranian mixed healthy dogs, 20 to 30 kg, one to five years old were used in this study in accordance with the 1996 guidelines of the Institute for Laboratory Animal Research (ILAR) of the National Academy of Science in Bethesda, MD, USA for the care and use of laboratory animals.

### Specimen Preparation

The material specimens were made into disks 8mm in diameter and 2mm thick. Four groups of specimens contained 12 samples in each group.

The Co-Cr alloy (Remaniun GM 900- Dentarum, Germany) disks were made using the "lost wax casting technique" according to manufacturer's instructions. The castings were first electrochemically polished in an ortophosphoric acid solution and then physically polished using a rubber wheel to create a satin-like finish.

The heat-cure acrylic resin specimens (Meliodent, Bayer UK Ltd., Berkshire, UK) were fabricated by investing wax patterns in stone molds within a dental flask as is done in actual denture processing. Packing and processing were carried out in accordance with the manufacturer's instructions (70°C, 9 hours). The acrylic specimens were finished and polished as is done with an actual acrylic resin denture base.

The specimens of the third group were fabricated by mixing heat-cure acrylic resin (Meliodent) and 20% of polymer by weight of $Al_2O_3$ powder (Martinswerk GmbH, Bergheim, Germany).

Teflon® disks were used for the control group. Teflon® and CR-CO alloy specimens were sterilized in a Siroclave 13 autoclave (Sirona, Verona, Italy) at 121ºC and a pressure of 15 psi for 15 minutes. The acrylic samples were sterilized with ethylene oxide gas.

### Implantation of Materials

In preparation for surgery the dogs were premedicated and systemically anesthetized using intramuscular injections of a mixture of 1.0 - 4 mg/kg Ketamine and 1.2 - 2.2 mg/kg Rampone followed by intubation of the animals.

The surgical sites were cleaned using 2% cholorhexidine solution and local anesthesia was administered. After the incision, a submucosal pocket was prepared by blunt dissection and the specimens were placed into the pocket above the periosteum followed by wound closure using surgical gut sutures.

Two specimens of each material randomly were implanted submucosally into one quadrant. One sample was implanted between the premolar and canine tooth and the other sample was implanted in the carnacia (4th premolar tooth of upper jaw and first molar tooth of lower jaw in a dog) area.

Other materials were implanted in other quadrants in each animal in the same way, but the sequence was rotationally changed for randomization purposes. After surgery, the animals were kept under care and soft diet during the first week.

Half of the specimens were removed under general anesthesia after 45 days and the other half after 90 days for histological examination. The biopsy tissue included 1 mm of connective tissues around the specimens. The specimens were fixed with a 10% buffered formalin solution then embedded in paraffin for processing. The paraffin blocks were cut in sections and mounted on glass slides then stained with hematoxylin and eosin. The state of the various types of inflammatory cells, occurrence, and location of fibrous tissue were graded 0-3 as shown in Table 1.

The sections were evaluated under a light microscope (Carl Zeiss MicroImaging GmbH, Bernried, Germany). Data were analyzed by Kruskal-Wallis, Mann-Whitney, and Tau b Kendal tests. The difference between the control and test groups was regarded as statistically significant ($P \leq 0.05$).

## Results

The degree of tissue reaction of the test groups and control group after 45 and 90 days is shown in Table 2. There was no significant difference in terms of the degree of tissue inflammation between the four groups at the two time intervals, 45 days ($P=0.330$) and 90 days ($P=0.292$), as indicated by the Kruskal-Wallis test. However, the degree of tissue inflammation between the four groups without consideration of time was close to the significance level ($P=0.089$) as determined by the Kruskal-Wallis test.

The Mann-Whitney test revealed a significant difference between the Co-Cr group and the control group ($P=0.021$). The degree of tissue reaction between the Co-Cr group and the acrylic resin group was close to the significance level ($P=0.078$).

Without considering individual groups there was no significant difference between the two test times ($P=0.144$). The results of this study showed

Table 1. Degree of tissue inflammation in connective tissue.[10]

| No inflammation (0) | Mild inflammation (1) | Moderate inflammation (2) | Severe inflammation (3) |
|---|---|---|---|
| No inflammatory cells | Presence of macrophages and/or plasma cells | Presence of macrophages and plasma cells | Focal areas of necrosis |
| More than 30 Fibroblasts | Less than 30 inflammatory cells | Occasional foci of neutrophil granulocytes and/or lymphocytes | Tissue densely infiltrated by inflammatory cells |
| Mature fibrous tissue, with many collagen | Between 10-30 Fibroblasts | More than 30 and less than 60 inflammatory cells | More than 60 inflammatory cells |
| | Immature fibrous tissue, with little collagen | 5-9 Fibroblasts | 1-4 Fibroblasts |

the degree of inflammation increased over time and mild inflammation changed to moderate or severe inflammation. The control group had no severe inflammation at any time (Table 2).

Although tissue inflammation between each group in the two time periods were analyzed using the Mann-Whitney test there was no significant difference ($P>0.05$) found.

Fibrotic capsule formation between groups was analyzed using the Kruskal-Wallis test without consideration for the time intervals of 45 or 90 days which showed significant differences between groups ($P=0.023$). The Mann-Whitney test revealed significant differences between the Co-Cr and the acrylic resin group ($P=0.011$) as well as the acrylic resin and control groups ($P=0.014$).

The acrylic resin group and acrylic resin mixed with $Al_2O_3$ group were close to the significance level ($P=0.065$). Other groups showed no significant differences ($P>0.05$). Time had no effect on fibrotic capsule formation ($P>0.05$).

The Tau-b Kendal test revealed correlation between fibrotic capsule formation and inflammatory reaction. Inflammation degree had significant and reverse relation with fibrotic capsule formation ($R=0.275$).

## Discussion

Inflammation is a response of living tissue to a local irritant, microbial infection, physical or chemical factors, or it can be an immunologic reaction. The inflammatory response surrounds and separates damaged tissue and inactivates microorganisms or toxins to facilitate tissue repair.[11] Evaluation of the biocompatibility of dental materials that come in contact with living tissue is critical. In the present study the biocompatibility of two current denture base materials and a suggested material for denture bases were assessed based on the presence of inflammatory cells, the fibroblastic reaction, and the severity of the inflammatory response over time. The Cr-Co alloy was more toxic compared with other materials. The alloy can corrode and release toxic elements. Some reports suggest such elements are more toxic than the original alloy.[12-14] There are studies showing the metals present in dental alloys can produce inflammatory effects.[15,16] Gulsen studied the effect of base metal alloys on human fibroblasts and reported Cr-Co and Cr-Ni alloys in sandblasted form are toxic.[9]

Another study reported high Nobel alloys are safer than nickel chromium (Ni-Cr) and Co-Cr.[17] In this study there was no statistical difference in inflammation between 45 days and 90 days but

**Table 2. Tissue reaction of the groups over time.**

| Material | | | | BIOCOPAM | | | Total |
|---|---|---|---|---|---|---|---|
| | | | | Mild inflammation | Moderate inflammation | Severe inflammation | |
| Co-Cr | Time | 45 days | Count | 3 | 1 | 2 | 6 |
| | | | % within time | 50.0% | 16.7% | 33.3% | 100.0% |
| | | 90 days | Count | 1 | 3 | 2 | 6 |
| | | | % within time | 16.7% | 50.0% | 33.3% | 100.0% |
| | Total | | Count | 4 | 4 | 4 | 12 |
| | | | % within time | 33.3% | 33.3% | 33.3% | 100.0% |
| Acrylic Resin | Time | 45 days | Count | 5 | 1 | 0 | 6 |
| | | | % within time | 83.3% | 16.7% | .0% | 100.0% |
| | | 90 days | Count | 3 | 2 | 1 | 6 |
| | | | % within time | 50.0% | 33.3% | 16.7% | 100.0% |
| | Total | | Count | 8 | 3 | 1 | 12 |
| | | | % within time | 66.7% | 25.0% | 8.3% | 100.0% |
| Acrylic Resin & $Al_2O_3$ | Time | 45 days | Count | 3 | 3 | 0 | 6 |
| | | | % within time | 50.0% | 50.0% | .0% | 100.0% |
| | | 90 days | Count | 3 | 2 | 1 | 6 |
| | | | % within time | 50.0% | 33.3% | 16.7% | 100.0% |
| | Total | | Count | 6 | 5 | 1 | 12 |
| | | | % within time | 50.0% | 41.7% | 8.3% | 100.0% |
| Control | Time | 45 days | Count | 5 | 1 | | 6 |
| | | | % within time | 83.3% | 16.7% | | 100.0% |
| | | 90 days | Count | 4 | 2 | | 6 |
| | | | % within time | 66.7% | 33.3% | | 100.0% |
| | Total | | Count | 9 | 3 | | 12 |
| | | | % within time | 75.0% | 25.0% | | 100.0% |

the severity of inflammation was increased from grade 1 to grade 2 and 3 over time. Remnants of the restorative material and the release of corroded metal elements from an alloy can explain the severity of inflammation. The same results have been reported by others.[16]

Some studies have described Cr-Co alloy as an inert material that can be implanted into bone without an inflammatory reaction.[18,19] Differences in alloy types, casting and polishing procedures, and the site of the implantation (hard or soft tissue) may affect tissue reaction. This study revealed the degree of tissue inflammation was more in the resin group compared to the control group but there was no statistical significant difference between them. Some studies have reported on the toxicity of acrylic resin.[4,20] Residual monomer and some chemical elements and pigments in acrylic polymer can induce inflammation.

Few studies have investigated the effects of adding $Al_2O_3$ filler as a thermal conductor on acrylic resins. The results of these investigations have shown ceramic filler can increase thermal conduction and improve some physical properties of acrylic resin.[6,8] The present study revealed the effect of acrylic resin mixed with $Al_2O_3$ filler specimens on tissue is similar to the acrylic resin group. The existence and the magnitude of increase in the inflammatory response in this group may be related to either the composition of the resin and fillers or to a foreign body effect.

In the control group mild to moderate inflammation was observed. The severity of inflammation increased over time. Teflon® (Poly Tetra Fluro-ethylene) is an inert material, and the presence of the inflammation might be related to foreign body reaction since local irritation can cause inflammation. Living tissue attempts to limit inflammation by enclosing or encapsulating it.

In the present study the severity of inflammation was increased but statistically there was no significant difference between test times (45 days and 90 days). Some studies have reported inflammation was reduced or remained constant over time.[21,22] This may be due to the biocompatibility of some materials and suitable reaction of the tissue to them. The present study revealed Co-Cr alloy has a more toxic effect on tissue than acrylic resin.

Inflammation in the acrylic resin mixed with $Al_2O_3$ group at day 45 was a little greater than the acrylic resin group but at day 90 the severity of inflammation was the same in both groups. It would appear $Al_2O_3$ particles were recognized as foreign bodies and tissue reaction to them was in the form of inflammation. Over time a fibrous capsule was formed to limit the inflammatory response. Although the acrylic resin group and the acrylic resin mixed with $Al_2O_3$ group showed more tissue reaction compared with the control group the difference was not significant. This might be related to acrylic composition, residual monomer, fillers, and pigments.

Acrylic resin mixed with $Al_2O_3$ is a suggested material for correction of thermal properties of acrylic resin. Previous studies showed improvement of mechanical properties and thermal conduction of the mixture.[6,8] This group had a lower tissue reaction compared to the Cr-Co group which is encouraging for future studies.

Fibrotic capsule formation was also assessed in this study. Fibrous capsule is an indication of good tissue reaction to the implanted materials because fibrous capsule formation separates foreign material from other tissue to minimize damage.[23]

In all groups fibrous capsules were formed at 45 days. The groups showed significant differences. The differences between the acrylic and Cr-Co groups, and the acrylic and control group were significant. The fibrous capsule in the acrylic group was formed more completely than in the other groups so grade 3 inflammations were not observed.

In the Cr-Co group fibrous capsules were formed to a lesser extent than other groups so the toxicity of Cr-Co is dominant and tissue repair is less.

In the control group the fibrous capsule was less well formed compared to the other groups because Teflon® is an inert material that had no toxic affect on the tissue. It only caused a foreign body reaction. Other studies have

supported the relationship between inflammation and fibrous capsule formation.[23] In the present study a significant reverse relationship between the degree of inflammation and fibrous capsule formation was seen.

## Conclusion

Within the limitations of this study it is concluded:

- Cr-Co alloy was more toxic compared to other groups.
- The acrylic resin and acrylic resin mixed with $Al_2O_3$ groups had more inflammation compared to the control group (no significant differences), but had less inflammatory reaction than the Cr-Co group.
- Inflammation and fibrous capsule formation had a significant inverse relationship.

## Clinical Significance

Co-Cr alloys are toxic and can produce damage to living tissue. Heat-cure acrylic resin materials are less toxic making them safer to use as a denture base than Co-Cr alloys.

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