The effects of recasting on the cytotoxicity of dental base metal casting alloys

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Abstract

Aim and Objectives: In dentistry, base metal casting alloys are extensively used for the fabrication of inlays, onlays, crowns, bridges, partial dentures, etc. During the casting of these alloys, excess amount of material used than needed will be collected as sprue buttons at the end, which is either added to the fresh alloy during casting and reused or disposed of.

Materials and Methods: The aim of the present in vitro experimental study was to investigate the effect of the complete recasting of four commercially available cobalt-chromium (Co-Cr) and nickel-chromium (Ni-Cr) base metal casting alloys on their cytotoxicity. During the study, four groups of alloys were subjected to complete recasting up to twenty times without the addition of new alloy. The cytotoxicity assessment of the selected alloys after recasting (Co-Cr and Ni-Cr alloys) was carried out using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay.

Results: The results indicated that Co-Cr alloys exhibit superior cell viability compared to Ni-Cr alloys, and cytotoxic potential of the alloys increased with repeated casting and led to increased cell death. The recasting of alloys in the present study did not show high cytotoxicity even after the 20th recasting.

Conclusion: From the results of the present study, it can be concluded that the alloys generated as a waste in the dental laboratory can be safely reused up to five times or at least once before they can be disposed, which reduces the cost of the treatment and also helps in conserving the natural resources.

Keywords: Base metal alloys; biocompatibility; cobalt-chromium alloys; cytotoxicity; nickel-chromium alloys; recasting; recycling

INTRODUCTION

Metallic materials play an essential role in the repair or replacement of the diseased or damaged tooth. The metals are more suitable for load-bearing applications due to their superior mechanical properties. However, the main disadvantage is the release of toxic ions, which results in various tissue reactions. The high costs of precious metals led to the development of various base metal alloys. Nickel-chromium (Ni-Cr) and cobalt-chromium (Co-Cr)-based alloys are the most commonly used for the fabrications of inlay, onlay, crowns, bridges, metal-ceramic restorations, cast posts, etc., in dentistry.1-4

The natural resources are getting depleted due to extensive usage.4-5 A recent study on recycling showed, reinforcing reused fibers enhanced the properties of the composite...
Recycling is the need of the hour for today’s life. Hence, every effort should be made to recycle and to keep the environment cleaner and greener. Recasting base metal alloys for about twenty times, without the addition of new alloys, has not disclosed any significant change in their mechanical properties. Previous research work of adding used alloys (50%–70%) with new alloys has not shown any changes in their properties. Geurtsen et al. reviewed corrosion and the release of metal ions from dental casting alloys and observed that Ni is the most frequent allergic element.

According to Chandra et al., the practice of reusing alloys in dental laboratories for economic reasons should be discouraged. Furthermore, recasting nickel-containing alloys with 65% surplus metal addition significantly increased the cytotoxicity. It is essential for practitioners to use their basic knowledge of the principles of biocompatibility to select the best dental casting alloys available. There is no evidence regarding what percentage of cast alloy can be mixed and how many times it can be reused. Base metal dental casting alloys used in a previous study showed that there was a slight decrease in mechanical properties (1st–20th recast), but this will not have any clinical significance. Mechanical properties are not only the prime factors to be considered for reusing these alloys, and the most important factor is biocompatibility.

Previous studies were not aimed at finding the maximum number of successive recycling operations without mixing fresh alloy before it becomes unsuitable due to cytotoxicity. The present study aims to investigate the effect of the complete recasting of four commercially available base metal casting alloys on their cytotoxicity without the addition of new alloy up to twenty times.

MATERIALS AND METHODS

This in vitro experimental study involved a total of four commercially available base metal dental casting alloys [Table 1]. The selected base metal alloys were cast into disc-shaped specimen of size 5 mm diameter and 3 mm thickness by lost wax casting process up to twenty times. To facilitate the casting procedure, the recasting was done using a cylindrical wax pattern using a new crucible. A total of 72 specimens (3 specimens of each alloy i.e., Manufacturer sample [MS], 1st Cast, 5th Cast, 10th Cast, 15th Cast and 20th Cast) were prepared for different alloys and deionized water was used as a control in the cytotoxicity test. The solution (deionized water) in which the alloys were incubated either for 24 hr, 48 hr or 1 week was added to the cells seeded in the wells to test the cell viability.

The cytotoxicity assessment of the selected alloys (Co-Cr and Ni-Cr alloys) was carried out using a well-known 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. This test enables screening of the materials for potential cytotoxicity effects on the cell cultures. The vero cells were seeded into a 96-well tissue culture plate. After 24 h, when partial monolayer formed, the medium was flicked off from the plate, and cells were treated with conditioned solution along with maintenance media for 48 h. After the treatment, the solutions in the wells were discarded, and 50 µl of freshly prepared MTT (2 mg/ml) was added to each well. The plates were shaken gently and incubated for 3 h at 37°C in a 5% CO₂ atmosphere. After 3 h, the supernatant was removed, and the formazan crystals formed in the cells were solubilized by the addition of 50 µl of isopropanol. Finally, the absorbance was read using a microplate reader at a wavelength of 540 nm using an ELISA microplate reader [Figure 2]. The percentage viability was calculated using the formula: percentage cell death = (OD control – OD sample/OD control) × 100.

Statistical analysis

The results obtained during the study were statistically analyzed using two-way ANOVA and Bonferroni test, and P < 0.05 is considered statistically significant.

RESULTS

Effect of recasting on cytotoxicity

In the present study, the cytotoxicity evaluation was carried out at different time intervals i.e., 24 h, 48 h, and 72 h by recasting up to twenty times. The cytotoxic effect was expressed in terms of cell viability, which indicates the number of cells that survived after the storage medium containing leached constituents of the alloy was added to the cell culture. The results of the cytotoxicity evaluation of various groups are presented in Figures 3-6, respectively.

The cytotoxicity evaluation of selected alloys revealed that the percentage of cell viability significantly changes when
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the alloy was first cast. Co-Cr alloys exhibit superior cell viability compared to Ni-Cr alloys. However, the percentage of cell viability was significantly affected when the alloy was recast, and it was reduced with each successive casting. The percentage of cell viability at the end of 1 week was always found to be less than the cell viability observed at the end of 24 h. Although both Co-Cr- and Ni-Cr-based alloys showed almost similar cytotoxicity results, the percentage cell viability of Co-Cr alloy was found to be slightly higher compared to Ni-Cr alloys.

**DISCUSSION**

The biocompatibility of the materials in contact with dental tissues plays essential role in their clinical acceptance.
Dental casting alloys have been used to fabricate various appliances and have a long history of dental use. To assess if the repeat casting of the alloy affects the biocompatibility, alloys were subjected to MTT assay, which is widely used as a screening test for various materials. In Ni-Cr alloys, despite a protective passivating layer, the release of nickel may lead to less cell viability. The difference in the amount of nickel release into the incubating solution may depend on the composition of the alloy. The composition of Ni-Cr ME-alloy is slightly different from Ni-Cr Wirolloy, and the percentage of nickel in ME-alloy is slightly less compared to Wirolloy [Table 1], indicating that the nickel release from ME-alloy may be less compared to Wirolloy. Further, the chromium content of ME-alloy is higher compared to Wirolloy, which may lead to better passivation in ME-alloy, which may reduce the metal ion release from these alloys. The results of the present study are in accordance with the previous investigation, which indicated that complete recasting of the alloys results in an increase of ion release and decreases cell viability. Yfantis et al. suggested that the enhanced corrosion behavior of Wirolloy was due to W addition and its relatively high Cr content. In the present study, Group I Wirolloy contains a higher amount of tungsten, which is not present in Group II Wironit alloys. In contrast, Co-Cr alloy compositions are considered highly stable due to the presence of a passivating layer of chromium.

The Co-Cr Wirolloy alloy used in the study showed less toxicity than the Co-Cr Wironit alloy. The difference in cell toxicity between Co-based alloys may vary due to the variation in the quantities of Cr [Table 1]. The Wirolloy alloy has higher Cr than Wironit alloy. Furthermore, the metal ion release from Wirolloy alloy was very low. Yfantis et al. suggested that the enhanced corrosion behavior of Wirolloy alloy was due to W addition and its relatively high Cr content. In the present study, Group I Wirolloy alloy contains a higher amount of tungsten, which is not present in Group II Wironit alloys. In contrast, a previous study indicated that the compatibility of Ni-Cr alloys was not significantly affected even after the alloy is recast five times. According to ISO (1999) 10993-5 standard, the degree of cytotoxicity is classified as noncytotoxic when cell proliferation is more than 75%, slightly cytotoxic when 50 to 75%, moderately cytotoxic when 25 to 50% and highly cytotoxic when less than 25%. The cytotoxicity of five times melted Group I and II Co-based alloys at 24 hours of testing was less than 25%, which can be considered as noncytotoxic (cytocompatible). At 48 hours and 1 week, it shows toxicity between 25 to 50%, i.e., less cytotoxic. Even after the 20th recasting, cobalt-based alloys were found to be less cytotoxic at different times. Ni-based alloys of group III and IV were less cytotoxic after 5th recasting, and in 15th and 20th recasting showed moderate toxicity. All the groups of alloys in the present study did not show higher toxicity values, i.e., 75% according to ISO.

In the present study, cytotoxicity of the selected alloy after repeat casting was measured at 24-h, 48-h, and 1-week intervals, which were not significantly different, though a decrease in the cell viability was observed with time. This indicates that the maximum ion release from the alloys incubated in water occurred within the first 24 h, and further release of metal ions into the incubating solutions was not very high. Overall, the present study indicates that the selected alloy cytotoxicity increases with repeat casting, which is attributed to the possible increase in the metal ion release.

**CONCLUSION**

A large amount of dental base metal casting alloys generated as scrap from dental laboratories is disposed without being recycled. The present study indicates that Co-based (Groups I and II) alloys can be recast up to five times and Ni-based alloys recast up to once without significantly affecting their toxicity. All the groups of alloys in the present study did not show high cytotoxicity. Efforts to be made to reuse these materials to reduce the cost of the treatment and save natural resources. If not, alternative methods to reuse these materials can be attempted since
these materials are also widely used for several engineering applications such as cutting tools.

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**Conflicts of interest**
There are no conflicts of interest.

**REFERENCES**

1. Anusavice KJ. Phillips’ Science of Dental Materials. 11th edition. WB Saunders. Philadelphia, 2003; page 103-5.
2. Gourav R, Ariga P, Jain AR, Philip JM. Effect of four different surface treatments on shear bond strength of three porcelain repair systems: An in vitro study. J Conserv Dent 2013;16:208-12.
3. Bacchi A, Dos Santos MB, Pimentel MJ, Caetano CR, Sinhoreti MA, Consani RL. Influence of post-thickness and material on the fracture strength of teeth with reduced coronal structure. J Conserv Dent 2013;16:139-43.
4. Nandish BT, Shenoy K, Shankarnarayana RK, Kukkila J Vaddya SB, Gingipalli K. Recycling of materials used in dentistry with reference to its economical and environmental aspects. IJHRS 2013;2:140-5.
5. Jayaprakash K, Kumar Shetty KH, Shetty AN, Nandish BT. Effect of recasting on element release from base metal dental casting alloys in artificial saliva and saline solution. J Conserv Dent 2017;20:199-203.
6. Jayaprakash K, Nandish BT, Rijesh M, Nayak J, Bhat SM, K Shetty KHK, et al. Fabrication of hair and copper fiber reinforced polymethyl methacrylate (PMMA) composites and evaluation of their mechanical properties, thermal conductivity, and color stability for dental applications. Trends Biomater Artif Organs 2016;30:8-12.
7. Jayaprakash K, Kumar Shetty KH, Shetty AN, Nandish BT, Rao S. Effect of recasting of Ni-Cr base metal dental casting alloys on the corrosion rate, compositional changes and ion release in artificial saliva and saline solution. IJHRS 2016;5:129-38.
8. Nandish BT, Shenoy K, Klaakkar RS, Jayaprakash K, Kishore G, Shama BV, et al. Evaluation of mechanical properties of recast dental base metal alloys for considering their reusability in dentistry and engineering field. Archives of Medicine and Health Sciences. 2014;2(2):178-183.
9. Mosleh I, Abdul-Gabbar F, Farghaly A. Castability evaluation and effect of recasting of ceramo-metal alloys. Egypt Dent J 1995;41:1357-62.
10. Al-Hiyasat AS, Darmani H. The effects of recasting on the cytotoxicity of base metal alloys. J Prosthet Dent 2005;93:158-63.
11. Geurtsen W. Biocompatibility of dental casting alloys. Crit Rev Oral Biol Med 2002;13:71-84.
12. Bajoghli F, Nosouhian S, Badriyan H, Goroochi H, Saberian A, Gadesi L. Effect of base metal alloys recasting on marginal integrity of castable crowns. J Contemp Dent Pract 2013;14:255-8.
13. Chandra TS, Kumar NS, Kumari BK. Evaluating cytotoxicity of recycled Ni-Cr dental casting alloys, an in vitro study. Trends Biomater Artif Organs 2011;25:51-9.
14. Imirzalioglu P, Alaaddinoglu E, Yilmaz Z, Oduncuoglu B, Yilmaz B, Rosenstiel S. Influence of recasting different types of dental alloys on gingival fibroblast cytotoxicity. J Prosthet Dent 2012;107:24-33.
15. Wataha JC. Biocompatibility of dental casting alloys: A review. J Prosthet Dent 2000;83:223-34.
16. Pujiyanto E, Siwomiharjo W, Ana ID, Tontowi AE, Wildon MW. Cytotoxicity of hydroxyapatite synthesized from local gypsum. BME Proc 2006;92-5.
17. Kumar NS, Chandra TS. Evaluation of variations in composition, corrosion behavior, and surface hardness on reusing a Co-Cr-Mo denture alloy. J Indian Prosthodont Soc 2008;8:22-6.
18. Yfantis C, Yfantis D, Anastassopoulos J, Theophanides T. Analytical and electrochemical evaluation of the in vitro corrosion behavior of nickel-chrome and cobalt-chrome casting alloys for metal-ceramic restorations. Eur J Prostodont Restor Dent 2007;15:33-40.
19. Poljak-Guberina R, Knezovic-Zlataric D, Katunaric M. Dental alloys and corrosion resistance. Acta Stomat Croat 2002;8:447-50.
20. International Organization for Standardization. ISO 10993-5: biological evaluation of medical devices - Part 5: Tests for in vitro cytotoxicity. Geneva: ISO; 1999.
21. Bilhan H, Bilgin T, Cakir AF, Yuksel B, Von Fraunhofer JA. The effect of mucine, IgA, urea, and lysozyme on the corrosion behavior of various nonprecious dental alloys and pure titanium in artificial saliva. J Biomater Appl 2007;22:197-21.
22. Yamamoto A, Honma R, Sumita M. Cytotoxicity evaluation of 43 metals salts using murine fibroblasts and osteoblastic cells. J Biomed Mater Res 1998;39:331-40.