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Effect of Recasting on Cytotoxicity of Ni-Cr Alloy - An In Vitro Study

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Abstract:
Background*: Cytotoxicity refers to the potential of a material to cause cell damage or death. In dentistry, cytotoxicity tests are essential to evaluate the biocompatibility of materials, especially when alloys are reused or recycled. During the casting and recasting process, minor elements in the alloy may be lost due to oxidation or volatilization, potentially altering the alloy's biological behaviour. Recasting can affect the release of metal ions, which may lead to adverse cellular responses. As such, cytotoxicity tests are used to assess whether recycled Ni-Cr base metal alloys remain safe for clinical use. These tests help determine whether the alloy still meets acceptable biological performance standards after being reused, and how many times it can be recast before becoming unsafe.* ***Materials and Methods****: In this in vitro study, A set of 25 samples was prepared for element release analysis to evaluate the types and quantities of elements released, which are critical for biocompatibility and toxicity. The samples were divided into five subgroups based on the proportion of recast to new alloy (100% new to 100% recast) to compare their impact on element release. The analysis was conducted using atomic absorption spectrometry (AAS)*
Results*: As the proportion of recast alloy increases, there is a significant rise in the release of elements like Nickel, Chromium, Molybdenum, Cobalt, and Copper, with the highest release observed in the 100% recast alloy group. Statistical analysis shows that these increases are highly significant ($p < 0.05$). This highlights the environmental and safety concerns associated with using recast alloys in material applications.* ***Conclusion:*** *Increasing recast alloy content significantly elevates the release of harmful elements, raising environmental and safety concerns.*
Key Word*:*

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I. Introduction

In dentistry, metals represent one of the four major classes of materials used for the reconstruction of decayed, damaged or missing teeth.[1] Base metal casting alloys are extensively used in dentistry to fabricate many oral appliances and a huge amount is wasted in the form of sprues and buttons during the casting procedure. Recycling and reusing these alloys by clean technologies may save our natural resources from being depleted and as well reduce the cost of the treatment of the patients.[1]

Base metal alloys contain more than75% of base metals (Co, Ni, Cr, Mo, Be, Al, Fe, Cu, W) and less than 25% noble metals. They generally exhibit superior corrosion resistance due to the formation of strong impervious surface oxide layer film, by a process known as passivation, superior mechanical properties like high modulus of elasticity, yield strength, tensile strength, surface hardness by solution and precipitation hardening and low density.[2] today, majority of all prosthetic restorations used clinically are of base metal alloy compositions. Several reports are available which recommend the use of 50% of previously cast metal alloy buttons or sprues removed from the castings.[3,4] Although the reasons for not reusing previously cast metal is not well documented, it is presumed that during the casting process certain important minor elements, present in small percentages in the original alloy compositions, may be lost during re-malting procedures through volatilization or oxidation. In addition, recasting of alloys has been shown to alter the properties of the alloys with respect to biocompatibility, mechanical properties and corrosion behavior.[5,6] Several reports are available regarding the effect of using previously cast alloys on their clinical performance. Some of these reports recommend the use of previously cast alloys and some of the report contradicts their use. In addition, little information is available about the number of times an alloy can be recast without losing its properties essential for their clinical performance[1.] This study was performed to explore new ways to recycle and reuse dental base metal casting alloys (Ni-Cr alloys) in dentistry by evaluating cytotoxicity.

II. Material And Methods

Study Design: in vitro study
Study Location: College Of Dental Science, Amargadh.
Study Duration: April 2023 to June 2024
Sample size: 25 samples
Sample size calculation: The sample size for each subgroup was determined based on the need for consistent comparison across varying proportions of recast to new alloy. Each group contains 5 samples to maintain uniformity and allow statistical analysis of trends or differences due to alloy composition. A total of 25 samples (5 per group × 5 groups) ensures adequate representation while balancing resource constraints.
The subgroups are classified based on the proportion of recast alloy to new alloy used. Here are the details of the subgroups:
Group 1:
Contains 5 samples.
This is the controlled group, consisting of 100% new alloy.
Group 2:
Contains 5 samples.
Composed of 25% recast alloy and 75% new alloy.
Group 3:
Contains 5 samples.
Composed of 50% recast alloy and 50% new alloy.
Group 4:
Contains 5 samples.
Composed of 75% recast alloy and 25% new alloy.
Group 5:
Contains 5 samples.
Composed of 100% recast alloy.

Procedure methodology
Fabrication of disc shaped specimen for element release analysis**:**

5 mm

3 mm

Wax Pattern Creation: The wax patterns for the five groups were created and segregated. These patterns were specifically designed for the disc-shaped specimens needed for the element release analysis.
Spruing: Each wax pattern was attached to a sprue, forming a pathway for the molten metal during the casting process.
Investing: The sprued wax patterns were invested in phosphate-bonded investment material within metal casting rings. Each casting ring was lined with dry cellulose paper ring liners to ensure a smooth and uniform mold surface.
Lost Wax Casting: The invested wax patterns were cast using the lost wax technique in an induction casting machine. The respective alloys were used for casting, sourced from cleaned leftover sprues and buttons of alloys previously cast in group I.

Cooling: After the casting process, the casting rings were allowed to bench cool to room temperature, ensuring the metals solidified properly within the molds.
Divesting and Sandblasting: The casting rings were carefully broken open to retrieve the cast metal specimens. These specimens were then sandblasted using aluminum oxide to remove any residual investment material.
Sprue Removal and Finishing: The sprues were cut off from the cast specimens. The specimens were then finished and polished using a sequence of carborundum discs, metal trimmers, rubber wheels, sandpapers, and polishing cake. Hand motor instruments were employed for precision finishing and polishing.
Cleaning: The polished discs were soaked in a detergent solution for 5 minutes, scrubbed with a soft bristle brush, and rinsed under tap water for another 5 minutes to ensure thorough cleaning.
Sterilization: The cleaned specimens were then autoclaved to ensure they were sterile and free from any contaminants.
Evaluation of Element Release
Incubation in Artificial Saliva: Discs of each alloy type from each group were transferred into 6 ml of artificial saliva. The specimens were incubated for 30 days at a constant temperature of 37°C to simulate the conditions of the oral environment.
Element Release Analysis: After the incubation period, the artificial saliva media was analyzed for the presence of various elements. The concentrations of nickel (Ni), chromium (Cr), molybdenum (Mo), cobalt (Co), and copper (Cu) released from the alloy specimens were measured.
Atomic Absorption Spectrometry: The analysis was conducted using atomic absorption spectrometry (AAS). This technique allowed for precise quantification of the elements released into the artificial saliva from the alloy specimens.

This detailed procedure ensured the accurate fabrication of disc-shaped specimens and provided reliable data on the release of elements from the different alloy types when exposed to conditions simulating the oral environment.

The data analysis reveals compelling insights into the effects of varying alloy compositions on the properties of the material under study.

One way ANOVA test:ANOVA (Analysis of Variance) is a statistical test used to analyze the differences between the means of three or more groups. It determines whether there are significant differences among group means and helps in understanding if those differences are due to actual effects or simply due to random chance. ANOVA assesses the variability between groups (due to the treatment or independent variable) relative to the variability within groups (due to random error).

Post hoc Tukey'stest: Post hoc tests are conducted following ANOVA when significant differences are found between groups. These tests further analyze pairwise comparisons between specific groups to determine which groups differ significantly from each other. Post hoc tests help in identifying where the differences lie when there are multiple groups involved in a study.

Level of significance: The level of significance (often denoted as α) in statistics represents the threshold below which the results of a statistical test are considered statistically significant. It indicates the probability of incorrectly rejecting the null hypothesis (which states there is no effect or difference) when it is actually true. Typically, a significance level of $\alpha = 0.05$ is used, meaning there is a 5% chance of incorrectly rejecting the null hypothesis. If a p-value from a statistical test is less than the significance level ($p < \alpha$), the results are deemed statistically significant, suggesting that the observed effects are unlikely to be due to chance alone

III. Result

The element release analysis conducted across groups with varying proportions of recast alloy demonstrates a clear and significant trend: higher proportions of recast alloy result in significantly increased release of elements, such as Nickel (Ni), Chromium (Cr), Molybdenum (Mo), Cobalt (Co), and Copper (Cu), compared to the control group with 100% new alloy.

For Nickel (Ni), Group 1 (100% new alloy) shows the lowest mean release at 0.1480 with a standard deviation of 0.00837. In stark contrast, Group 5 (100% recast alloy) exhibits a mean release of 0.7520 with the same standard deviation, indicating a substantial increase in nickel release. The F-value for this comparison is exceptionally high at 3218.71, with a p-value of 0.01, indicating a statistically significant difference across the groups.

Similarly, Chromium (Cr) release is markedly higher in Group 5 compared to Group 1. Group 1 has a mean chromium release of 0.1020 (std. deviation = 0.00837), whereas Group 5 has a mean release of 0.7020 (std. deviation = 0.00837). The F-value for chromium release stands at 3966.42, and the p-value is 0.01, further confirming the significant differences between the groups.

For Molybdenum (Mo), Group 1 exhibits a mean release of 0.0500 with a standard deviation of 0.00707, while Group 5 shows a mean release of 0.2980 with a standard deviation of 0.00837. The F-value here is 697.97, with a p-value of 0.01, underscoring the significant increase in molybdenum release as the proportion of recast alloy rises.

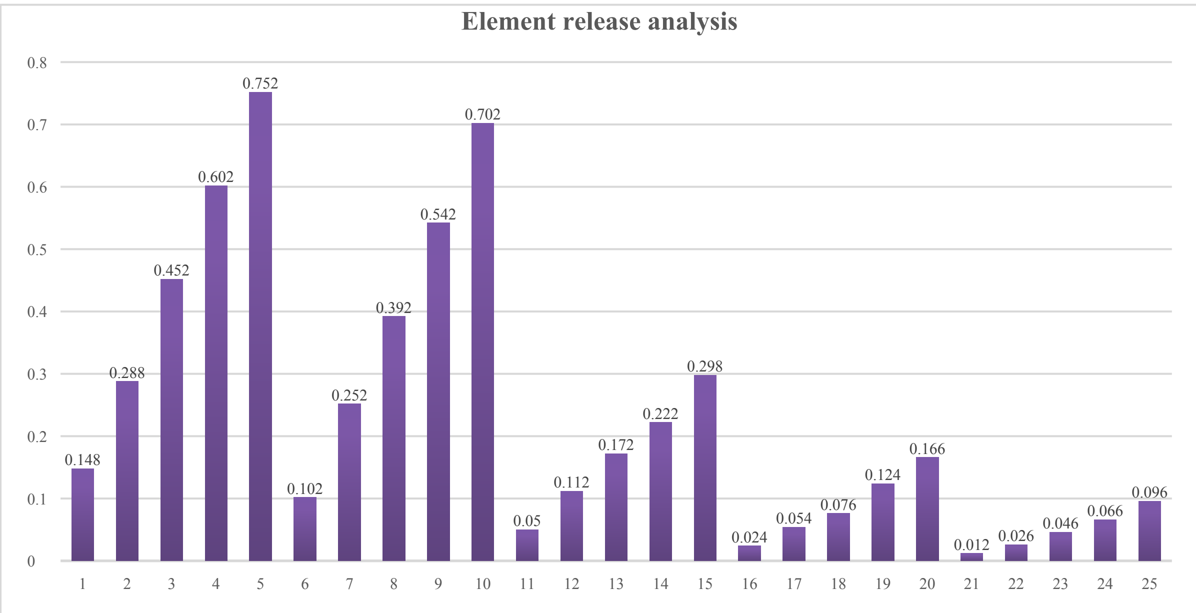
In the case of Cobalt (Co), the mean release in Group 1 is 0.0240 (std. deviation = 0.00548), compared to a much higher mean release of 0.1660 in Group 5 (std. deviation = 0.00548). The F-value for cobalt release is 532.20, with a p-value of 0.01, indicating a statistically significant difference between the groups.

Finally, Copper (Cu) release follows the same pattern, with Group 1 showing a mean release of 0.0120 (std. deviation = 0.00447), and Group 5 showing a mean release of 0.0960 (std. deviation = 0.00548). The F-value for copper release is 196.64, and the p-value is 0.01, confirming the significant differences across the groups.

In summary, the element release analysis unequivocally demonstrates that higher proportions of recast alloy lead to significantly increased release of Ni, Cr, Mo, Co, and Cu. Each element shows a progressive increase in mean release values from Group 1 (100% new alloy) to Group 5 (100% recast alloy), with all differences being statistically significant. This trend highlights the potential biocompatibility and toxicity concerns associated with using higher proportions of recast alloy in materials, emphasizing the need for careful consideration of alloy composition in applications where element release is a critical factor.

Table no 1 : Element Release Analysis

		N	Mean	Std. Deviation	F-Value	p-Value
Ni	GE1	5	.1480	.00837		
	GE2	5	.2880	.01304		
	GE3	5	.4520	.00837	3218.71	0.01*
	GE4	5	.6020	.00837		
	GE5	5	.7520	.00837		
Cr	GE1	5	.1020	.00837		
	GE2	5	.2520	.00837	3966.42	0.01*
	GE3	5	.3920	.00837		
	GE4	5	.5420	.00837		
	GE5	5	.7020	.00837		
Mo	GE1	5	.0500	.00707		
	GE2	5	.1120	.00837	697.97	0.01*
	GE3	5	.1720	.00837		
	GE4	5	.2220	.00837		
	GE5	5	.2980	.00837		
Co	GE1	5	.0240	.00548		
	GE2	5	.0540	.00548	532.20	0.01*
	GE3	5	.0760	.00548		
	GE4	5	.1240	.00548		
	GE5	5	.1660	.00548		
Cu	GE1	5	.0120	.00447		
	GE2	5	.0260	.00548	196.64	0.01*
	GE3	5	.0460	.00548		
	GE4	5	.0660	.00548		
	GE5	5	.0960	.00548		



IV. Discussion

Base-metal alloys are used extensively in dentistry. Cast Ni-Cr and Co-Cr alloys are used in ceramic metal restorations. Ni-Cr alloys containing Be are still popular.[7] The attractiveness of these materials stems from their corrosion resistance, high strength and modulus of elasticity, low density, and low cost.[7,8] These alloys are harder than noble alloys but usually have lower yield strengths.[7,8] The physical properties of these alloys are controlled by the presence of minor alloying elements such as carbon, molybdenum, beryllium, tungsten, manganese, nitrogen, tantalum, gallium, and aluminum.[7] Many Ni-Cr alloy formulations contain up to 2% by weight of beryllium. The major reason for incorporating this element in the alloy is to lower the melting range and to decrease the viscosity of the molten alloy, thereby improving its castability.[9] Some Ni-Cr alloys, especially those containing Be, have mold-filling abilities superior to all other groups. This mold-filling permits easier casting of thin sections and produces sharp margins on castings.[10] These base metal casting alloys are generally considered more technique sensitive and difficult to cast than noble casting alloys.[9] Allergic responses to the constituents of base metal alloys, especially Ni, are observed occasionally. High hardness complicates occlusal adjustment and polishing.[8]

These alloys are carefully formulated by the manufacturers and supplied as pellets are melted and cast into a mold prepared by a lost wax casting process, a well-established and routinely practiced technique in the dental laboratories. During the process of casting of these alloys, an additional amount of alloy than required for making the restoration is used to compensate for the alloy shrinkage and to make up for the volume of sprue buttons and reservoir. After the casting process, the spruebuttons are cut off from the alloy restoration leading to the generation of lot of alloy as scrap or waste. In order to reduce the cost of the treatment and to save the metal alloy, it has been a general practice in most dental laboratories to reuse the scrap or waste. Even the suppliers of dental casting alloys recommend such practice wherein about 50% of fresh alloy is used along with the scrap during repeat casting process, though the exact reason for such practice is not clear. However, it is not clear if the scrap that is generated with only 50% fresh alloy can again be reused.[11,12,13,14]

Several research investigations have been carried out to assess the effect of reusing scrap on the properties of the base metal casting alloys. The existing knowledge on the use of scrap metal during the repeat casting of dental alloys indicate that recasting effects the properties of alloys which depend specifically on the alloy composition, the method of casting and variations in testing methodology. The variations in composition of the alloys and testing methodologies reported in the literature are so diverse that comparison between the studies is difficult[15,16,17].

In addition, it is not clear if any given alloy composition can be completely reused for dental applications and if not, the reasons for the same have not been reported so far in the literature. In this backdrop, the present study was designed to answer the following questions:

- Can the dental base metal alloys be recast for several times before they become unusable?
- What are the effects of changes in elemental release of the alloy?

Biocompatibility of the materials that are placed in contact with dental tissues plays an important role in their clinical acceptance. Dental casting alloys have been used for the fabrication of various appliances and have a long history of dental use. At the same time, some compositions of dental alloys are known to be allergenic or toxic in some instances. The most common elements that are known to be allergenic or toxic are nickel and beryllium. Nickel is known to be the most allergic metal with incidence of allergy ranging from 10 to 20%. Beryllium, on the other hand, is known to be highly toxic. It is used in dental casting alloys in about 1 to 2 % (weight) to improve the castability of the alloys. It is also presumed to help in metal ceramic bonding by forming an oxide layer. No studies have been reported in the literature which indicates that dental alloys containing beryllium caused cancer in humans. However, beryllium in dental casting alloys is known to cause Berylliosis, a condition which occurs due to the chronic inhalation of dust containing beryllium, especially for personnel involving in manufacturing and fabrication process.

The allergic or toxic potential of these metals depend on their release into the surroundings. Since the repeated casting of the base metal alloys investigated in the present study showed changes in the composition, it is assumed that it will have an effect on the biocompatibility of these alloys as well. In order to assess if the repeat casting of the alloy affects the biocompatibility, the alloys were subjected to AAS assay which is widely used as a screening test for various materials

The selected base metal alloys based on Nickel-Chromium were cast into disc shaped materials. These disc shaped specimens were incubated in artificial saliva which was used to test the element release. The purpose of incubating alloy discs in artificial saliva is to extract metal elements into the solution which is the case in oral condition.

Commercially available base metal alloys for fixed partial dentures are usually Ni–Cr alloys [1, 2]The composition of these alloys may vary according to manufacturer but the basic constituents are Ni, Cr and Mo. Addition of minor components significantly changes the microstructure and properties which affect the bond strength of ceramic to metal oxide layer required for achieving bonding. It is at the manufacturer's discretion to include any of these minor components to produce the desired properties.

The findings of the current study agreed with the hypothesis that recasting of base metal alloys increases their potential cytotoxic effect and element release. In the study, element release in all the new alloy samples was significantly less than the re-casted specimens.

It has been reported in the literature that the major contribution for toxicity of alloys was probably due to copper content. It has been reported that only Ni–Cr alloys containing 16–27% Cr develop an adequate protective oxide layer. Molybdenum also has an active role in the formation of oxide layer. Since copper based alloys tarnish and corrode more than non-copper alloys, it was observed in the present study that a remarkable amount of copper was released into culture medium from 100% recast alloy. The content of copper in association with low content of Cr and absence of molybdenum may also explain significant cytotoxic effect of this alloy in comparison. Thus for all of the alloys 50% recast groups were significantly more cytotoxic than 100% new alloy groups and furthermore, the 100% recast groups were significantly more cytotoxic than 50% recast group.

From the results of present study it appears that the alloy types and the elements of these alloys become affected to a different degree when the alloy was recast and hence attributes to its cytotoxic effects.

The results of the present study are in accordance with the previous research investigation which indicated that complete recasting of the alloys result in increased element release and thus increased cytotoxicity.

Limitations

The results of the study need to be correlated considering the following limitations.

- The observed results from the present study may not exactly simulate the oral conditions However, the observed results will help in understanding the effect of recasting on the cytotoxicity of the alloys.
- The minerals or organic constituents in artificial saliva used may have an effect on corrosion susceptibility of the alloys and thus influence the element release.
- Although a small sample size was used in the current study, significant differences were found between the different groups, indicating its sufficiently large effect size. However further studies need to be conducted with greater sample size for a more adequate representation of the alloy types.

V. Conclusion

The undesirable property of the repeatedly recast alloys is the cytotoxicity and small changes in the composition. Changes in physical properties are trivial and do not affect its reuse. Though, the present study provides sufficient data for the assessing the effect of recasting on the properties of base metal alloys, further research in terms of selecting large number base metal alloy compositions, effect of recasting on corrosion susceptibility of the alloys and the further assessment of biological properties of these alloys may help in further understanding of the effect of recasting on the properties of base metal casting alloys.

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