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Effects of surface finishing conditions on the biocompatibility of a nickel–chromium dental casting alloy

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ABSTRACT

Objectives. To assess the effects of surface finishing condition (polished or alumina particle air abraded) on the biocompatibility of direct and indirect exposure to a nickel–chromium (Ni–Cr) d.Sign®10 dental casting alloy on oral keratinocytes. Biocompatibility was performed by assessing cellular viability and morphology, metabolic activity, cellular toxicity and presence of inflammatory cytokine markers.

Methods. Discs of d.Sign®10 were cast, alumina particle air abraded and half were polished before surface roughness was determined by profilometry. Biocompatibility was assessed by placing the discs directly or indirectly (with immersion solutions) into contact with TR146 monolayers. Metal ion release was determined by ICP-MS. Cell viability was assessed by trypan blue dye exclusion, metabolic activity by XTT and cellular toxicity by LDH. Inflammatory cytokine analysis was performed using sandwich ELISAs.

Results. The mean polished Ra value was significantly reduced ($P < 0.001$) compared with the alumina particle air abraded discs but metal ion release was significantly increased for the polished discs. Significant reductions in cell density of polished compared with alumina particle air abraded discs was observed following direct or indirect exposure. A significant reduction in metabolic activity, increase in cellular toxicity and an increase in the presence of inflammatory cytokine markers was highlighted for the polished relative to the alumina particle air abraded discs at 24 h.

Significance. Finishing condition of the Ni–Cr dental alloy investigated has important clinical implications. The approach of employing cell density and morphology, metabolic activity, cellular toxicity levels and inflammatory marker responses to TR146 epithelial cells combined with ICP-MS afforded the authors an increased insight into the complex processes dental alloys undergo in the oral environment.

1. Introduction

Non-precious nickel (Ni)-based dental casting alloys possess a high modulus of elasticity which enables use in thinner sections than conventional high-gold alloys, which make Ni-based alloys ideal for a variety of applications in restorative dentistry [1]. As Ni-based dental restorations are in direct, prolonged contact with the gingival tissues, often extending subgingivally [2], the long-term prognosis of these alloys to cause a concern to oral health cannot be overlooked. Metal ion concentrations and corrosion products leached from den-

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tal alloys into the adjacent gingival tissues have been reported [3] and proposed to be dependent upon the bulk composition of the alloy which influences the corrosion resistance [4–12], the microstructure formed during the casting procedure [2] and subsequent firing protocols [1,2,13,14].

Corrosion occurs with associated metal ion release from the restoration into adjacent gingival tissues [3] or alternatively by the progressive dissolution of a surface film which results in the metal being totally consumed which results in the metal being totally consumed by the reaction (oxidation) or the formation of a protective passivation layer (reduction) [7,13]. Disruption of the protective passivation oxide layer can be caused by a number of different mechanisms including anodic dissolution whereby the passive layer undergoes a process of partial dissolution and reprecipitation in the aqueous solution [11]. Corrosion can occur as pitting, crevice and galvanic processes which can subsequently manifest in Ni-based dental alloys *in situ* in the oral cavity [2]. Biological factors including decreased pH disruption [15], the presence of active oxygen species [16,17] and the acceleration of leaching by presence of amino acids and proteins [18,19] can also further exacerbate corrosion processes. As a result, the elemental components of these alloys and possibly any associated corrosion products leached into the surrounding gingivae during function have the potential to cause hypersensitivity [5].

Nickel is a potent allergen and causes hypersensitivity reactions to a greater extent compared with any other metal or alloys used in metal-ceramic restorations with approximately 20% of women and 2% of males between the ages of 16 and 35 years susceptible to nickel sensitivity [20]. Metal ion release can be directly linked to the clinical side effects observed with the use of nickel–chromium (Ni–Cr) dental casting alloys in the oral cavity. Inflammatory responses associated with Ni–Cr alloy restorations subside when the alloy is removed and replaced with nickel-free alloy alternatives thereby providing evidence to this issue.

It should be noted that the majority of publications in the dental literature focus on the relationship between metal salts of the major constituents of Ni–Cr dental casting alloys and oral epithelial cells [3,4,6,16,17,21–24]. As a result, the clinical relevance to dentistry of the relationship between metal salts and oral epithelial cells is difficult to translate. The authors of the current study therefore believed that physical presence of a dental casting alloy (through direct contact or indirect contact with immersion solutions) to oral keratinocytes was a pre-requisite to provide an accurate, repetitive and realistic clinical portrayal of metal ion release through the corrosion behavior of Ni–Cr alloys.

The study therefore focused on whether the surface finish condition of a Ni–Cr dental alloy had the potential to modify cell density (using the trypan blue dye exclusion assay), cell morphology, metabolic activity (using tetrazolium based 2, 3-Bis (2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide inner salt sodium assay (XTT)), cellular toxicity levels (using the release of the cytosolic enzyme lactate dehydrogenase (LDH)) and inflammatory marker (Interleukin-1α (IL-1α), PGE_2 (Prostaglandin E_2) and Tumor Necrosis Factor-α (TNF-α)) responses to TR146 epithelial cells using a sandwich Enzyme Linked Immunosorbent Assay (ELISA).

The influence of surface finishing condition has rarely been investigated in the dental literature [2,7,13,18], with a surface finishing condition equivalent to that used clinically seldom used for biocompatibility evaluations [2]. The majority of studies that investigated the biocompatibility of Ni-based dental casting alloys were performed on metal salt solutions [4,16,21,23,24] rather than the cast alloy in the surface finish equivalent to the clinical condition [2]. According to Roach et al. [1] polishing should allow for the uptake of atmospheric oxygen by the exposed surface, thereby acting as a 'nonconductive barrier' to electron flow. The surface finishing condition of the Ni-based dental casting alloy was therefore considered to be a critical factor based on the 'nonconductive barrier' postulated by Roach et al. [1].

Novel methodologies employed for the assessment of ion release for Ni–Cr dental casting alloys also include Laser-Ablation Inductively Coupled Plasma-Mass Spectrometry (LA-ICPMS) [5], Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES) [2] and ICP-MS used in the current study. While LA-ICPMS can assess the distribution of ions in tissues, both ICP-AES and ICP-MS can assess ion release in immersion solutions with detection limits of concentrations of 0.04 μg/mL and below one part in 10^{12}, respectively and therefore ICP-MS was chosen in the current study.

The aim of the current study was to assess the influence of surface finishing condition (polished or alumina particle air abraded) on the biocompatibility of direct and indirect exposure of a commercially available Ni–Cr dental casting alloy (d.Sign®10, Ivoclar Vivadent, Leicester, UK) on a TR146 immortal human keratinocyte cell line. Biocompatibility was analyzed by the assessment of TR146 cell density and cell morphology using light microscopy and cell viability using a trypan blue dye exclusion assay. Cellular proliferation analysis was performed using an XTT metabolic assay, cellular toxicity levels were determined with an LDH assay and metal ion release by ICP-MS was also performed. Immunological cytokine profiles with a sandwich ELISA method specific for the inflammatory molecules IL-1α, IL-8, PGE_2 and TNF-α in response to the alloy finishing conditions when exposed directly (to the alloy) or indirectly (to the immersion solutions) were also assessed.

2. Materials and methods

2.1. *Materials*

A commercially available Ni–Cr dental casting alloy (d.Sign®10) was employed. The main constituents of the alloy (in mass%) as reported by the manufacturers were 75.4% Ni, 12.6% Cr and 8% Mo and the alloy indications included the production of crowns, short and long spans bridges, posts and telescope crowns [25]. Disc-shaped specimens (15 mm diameter and 1.0 mm thickness) were prepared from wax patterns (Blue inlay casting wax, Kerr Italia SpA, Salerno, Italy) connected by a 3 mm diameter sprue (Dentaurum, Turnstrase 31, Ispringen, Germany) to the sprue former (Whip Mix™, Kentucky, USA) and positioned in the center of the casting ring (Whip Mix 4088, Whip Mix™, Kentucky, USA) resulting in a 5 mm sprue length. A carbon-free phosphate-