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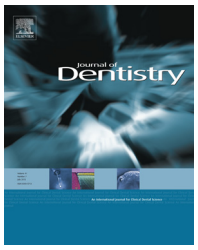

Biocompatibility effects of indirect exposure of base-metal dental casting alloys to a human-derived three-dimensional oral mucosal model

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ARTICLE INFO

Article history:
Received 11 June 2013
Received in revised form
6 August 2013
Accepted 7 August 2013

Keywords:
Biocompatibility
Dental casting alloys
Cytotoxicity
Inflammation
Tissue engineering

ABSTRACT

Objectives: The study employed a three-dimensional (3D) human-derived oral mucosal model to assess the biocompatibility of base-metal dental casting alloys ubiquitous in fixed prosthodontic and orthodontic dentistry.

Methods: Oral mucosal models were generated using primary human oral keratinocyte and gingival fibroblast cells seeded onto human de-epidermidised dermal scaffolds. Nickel–chromium (Ni–Cr) and cobalt–chromium (Co–Cr) base-metal alloy immersion solutions were exposed to oral mucosal models for increasing time periods (2–72 h). Analysis methodologies (histology, viable cell counts, oxidative stress, cytokine expression and toxicity) were performed following exposure.

Results: Ni-based alloy immersion solutions elicited significantly decreased cell viability ($P < 0.0004$) with increased oxidative stress ($P < 0.0053$), inflammatory cytokine expression ($P < 0.0077$) and cellular toxicity levels ($P < 0.0001$) compared with the controls. However, the Ni-free Co–Cr-based alloy immersion solutions did not elicit adverse oxidative stress ($P > 0.4755$) or cellular toxicity ($P < 0.2339$) responses compared with controls.

Conclusions: Although the multiple analyses highlighted Ni–Cr base-metal alloy immersion solutions elicited significantly detrimental effects to the oral mucosal models, it was possible to distinguish between Ni–Cr alloys using the approach employed. The study employed a 3D human-derived full-thickness differentiated oral mucosal model suitable for biocompatibility assessment of base-metal dental casting alloys through discriminatory experimental parameters.

Clinical significance: Increasing incidences of Ni hypersensitivity in the general population warrants serious consideration from dental practitioners and patients alike where fixed prosthodontic/orthodontic dental treatments are the treatment modality involved. The novel and analytical oral mucosal model has the potential to significantly contribute to the advancement of reproducible dental medical device and dental material appraisals.

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0300-5712/$ – see front matter
http://dx.doi.org/10.1016/j.jdent.2013.08.010

1. Introduction

Recent epidemiological data suggests over 65 million people in Europe have undergone at least one reaction to nickel (Ni) in their lifetime.[1] In dentistry, Ni-based base-metal dental casting alloys account for approximately 80% of fixed prosthodontic restorations[2] with Ni-based base-metal wires and brackets routinely being used in orthodontic treatment.[3,4] Oral exposure to Ni has been definitively shown to induce dermatitic flares in Ni-sensitised persons.[5,6] In addition, Ni hypersensitivity reactions from the leaching of metallic elemental components into the surrounding oral mucosal tissues have been identified to be associated with Ni-based fixed prosthodontic[2] and orthodontic[3,4] dental appliances. Ni hypersensitivity reactions are attributed to a base-metal dental casting alloy's ability to corrode in vivo which is dependent upon the alloy phase structure,[7] alloy composition[8] and the biological environment[9] to which it is subjected. However, the challenging oral environment (temperature variations,[10] extended life spans,[11] bacterial interference[12]) in which base-metal dental restorative appliances exist during function suggest a level of metal ion leaching is unavoidable.[13]

The biocompatibility potential of dental materials are habitually assessed using two-dimensional (2D) cell monolayer structures[14,15] in line with the International Organization for Standardization (ISO)[16] concerning biocompatibility evaluations of dental medical devices. Previous translational toxicity models employed animals which are limited by ethical and biological concerns. Additionally, 2D cell monolayer structures bear little physical resemblance to complex 3D tissues,[17] leading to increased toxin susceptibility,[18] unsuitable immune reactions,[19] thereby limiting their clinical application and relevance. Murine models have also been employed for dental device biocompatibility appraisal,[20,21] however, intra-species variation in condensed Toll-like receptor 4 (TLR4) regions have been identified,[1] rendering murine models unsuitable for future Ni hypersensitivity studies.

The principle of a tissue-engineered 3D oral mucosal equivalent is that it should imitate the native human oral mucosal structure through an upper stratified squamous epithelium with underlying dense lamina propria.[22] Progress in tissue engineering has led to the development of oral mucosal equivalents employing Alloderm™ as a biodegradable scaffold material, due to its increased mechanical properties compared with synthetic scaffolds.[23–26] Previous attempts at creating oral mucosal equivalents have utilised either murine[23,24] or immortalised human cells.[26] The TR146 oral keratinocyte cells used by Moharamzadeh et al.[25,26] were advantageous over murine cells[23,24] due to their human origin, however, as immortalised cells, the TR146 oral keratinocytes were limited by their inherent lack of differentiation abilities. The use of primary human oral cells (oral keratinocytes and gingival fibroblasts) in association with the human-derived Alloderm™ scaffold has been advocated recently to generate an oral mucosal model.[27] The authors suggested the human-derived oral mucosal model could for the first time be considered an accurate representation of the native human oral mucosal structure with associated enhanced biological and immunological responses for dental medical device biocompatibility analyses.

Following direct exposure of human-derived oral mucosal models to Ni-based base-metal dental casting alloys, the deleterious effects of Ni ions were considered symptomatic of hypersensitivity reactions routinely observed clinically in oral tissues adjacent to Ni-based base-metal restorations.[27] However, the potential for Ni-based base-metal dental casting alloys to induce hypersensitivity reactions in the oral cavity is not limited to the local area of placement. The proximity of the oral mucosa comprising masticatory, lining and specialised mucosal linings,[28] to a given Ni-based base-metal restoration, is increased exponentially through salivary distribution.[7]

Therefore, the aim of the current study was to assess the biocompatibility of base-metal dental casting alloys following indirect exposure to 3D human-derived oral mucosal models. Biocompatibility was assessed through histology, viable cell counts, oxidative stress responses, inflammatory cytokine expression and cellular toxicity. Accordingly, the objective was to establish the biocompatibility potential of base-metal dental casting alloys following indirect exposure to human-derived full-thickness 3D oral mucosal models.

2. Materials and methods

2.1. *Alloy-disc preparation*

Three base-metal dental casting alloys (two Ni–Cr and one Co–Cr, Table 1) were cast to form alloy-discs (10.0 ± 0.1 mm diameter and 1.0 ± 0.1 mm thickness) in accordance with the manufacturer's recommendations.[29–31] The alloy-discs were divested by alumina particle air abrasion (Renfert Basic Master, Buckinghamshire, UK) using 50 μm aluminium oxide at 2 bar pressure at a distance of 5 cm for 20 s, separated from the sprues using a cutting disc and prepared to a clinical surface finishing condition with rubber polishing wheels.[8,32] The base-metal alloy-discs were sterilised at 115 °C for 15 min using an autoclave (LTE Touchclave-LAB, LTE Scientific Ltd., Oldham, UK).

Table 1 – Composition (% wt.) of the base-metal dental casting alloys employed in the current study as provided by the manufacturer Ivoclar Vivadent, Schaan, Lichtenstein.[29–31]

	Ni	Cr	Mo	Co	Al	Nb	Si	Fe	Ga
d.Sign®10	75.4	12.6	8.0	–	3.3	–	<1.0	–	–
d.Sign®15	58.7	25.0	12.1	<0.1	–	–	1.7	1.9	–
d.Sign®30	–	30.1	<1.0	60.2	<1.0	3.2	<1.0	<1.0	3.98