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The effect of a microcarrier suspension cell culture system on polarization measurements from Ni–Cr dental casting alloys

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Summary *Objectives*. Recent research has demonstrated that cells/cellular components can influence the corrosion or degradation of the implant material in addition to being challenged by the cytotoxic by-products the implant material may release. The overall objective of this research was to modify a microcarrier suspension cell culture system to incorporate an active corrosion experimental capacity.
Methods. The ability to conduct polarization experiments on two Ni–Cr dental casting alloys under the following environmental conditions: media only, media plus serum, media plus serum and antibiotics (complete media), complete media with microcarriers, and complete media with cells grown on microcarriers; was evaluated during this initial study.
Results. Results obtained were reproducible within sample groups (95% confidence level) indicating the precision of the corrosion set-up under all environmental conditions. These studies also show that media with serum and antibiotics (complete media) induced a significantly higher corrosion rate (95% confidence level) for both materials compared to the other test conditions.
Significance. Future experiments will focus on cytotoxic effects caused by parametrically controlled corrosion experiments on the suspension cell cultures, including co-cultures.

Introduction

Nickel-based alloys have been in use since 1930 [1,2]; however, there are concerns regarding the corrosion or degradation by-products these

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alloys release into the surrounding tissue and their cytotoxicity to the tissue's normal function. Clinically, nickel released from these alloys has been shown to cause adverse tissue reactions and prosthetic loosening [3–7]. Although several studies have reported normal morphology, ultrastructure, viability, and DNA synthesis [8–15], others have reported decreases in DNA synthesis and inhibition of various enzymes of cultured cells when exposed to nickel-based alloys [16–19].

The nickel–chromium (Ni–Cr) alloys used in this study include Neptune and Vera Bond. The chemistry, microstructure, surface composition, and corrosion behavior of these casting alloys have been evaluated and previously published [20]. Bumgardner et al. [20] reported that corrosion rates for Neptune were lower than for the beryllium containing alloys, including Vera Bond. According to atomic absorption studies, the concentration of ions released into media without cells present was not proportional to the composition of the alloys. Human gingival fibroblasts exposed to the alloys exhibited alterations in proliferation, glucose-6-phosphate-dehydrogenase activity, and intracellular ATP levels. SEM and TEM evaluations of the cells exposed to the nickel alloys displayed no alterations in morphology [19].

While these reported studies have revealed possible concerns with the cellular response to nickel-based alloys, the ions they release are also a concern [21]: such as alterations in enzyme activities; ATP levels, and DNA, RNA, and protein synthesis [9]. To investigate the effects of the ions released, several studies including Schmalz et al. and Wataha et al. [22,23] exposed fibroblasts to different extracts produced from alloys. However, metallic ions released in the extracts may react with the proteins and salts in the extract solution altering their cytotoxicity over time [21,22]. Therefore, using the alloy is the best way to determine its cytotoxicity and the cytotoxicity of the degradation by-products it releases to surrounding tissues and systemic tissues.

Long-term cellular responses have been estimated by the use of extracts of corrosion products from passive dissolution studies, but again the metallic ions released alter chemistry and activity by interacting with salts and proteins in the extract media over time. In addition, research from as early as the 1980s by Brown and Merritt, has demonstrated that cells/cellular components can influence the corrosion or degradation of the implant material in addition to being challenged by the cytotoxic by-products the implant material may release [24].

Thus, the overall objective of this research was to modify a microcarrier suspension cell culture system to incorporate an active corrosion experimental capacity. Incorporation of such active corrosion experimental capacity will allow for control and monitoring of: (1) the amount of cytotoxic by-products released by the implant material, and (2) the chemical species released by the implant material. The current study is a first step in developing this complex system and will validate the effects of each component of the test solution (media, serum, antibiotics, microcarriers, and cells) on the capacity to conduct precise and repeatable polarization experiments two Ni–Cr dental casting alloys.

Materials and methods

Alloy preparation

The nickel–chromium alloys used for these studies were Neptune and Vera Bond; their compositions can be found in Table 1 [19]. Alloys were polished with a hard blue rubber wheel (Dodoeco International, Inc., Long Eddy, NY), a white flexxie (Dodoeco International, Inc., Long Eddy, NY), a pink rubber wheel, and a No. 11 Abbott–Robinson soft bristle brush (Buffalo Dental Mfg Co., Inc., Syosset, NY) with Chrometal burnishing Agent No. 2 (The Motloid Co., Chicago, IL) [20]. The alloys were measured, ultrasonically cleaned in ammonium hydroxide for 5 min and sterilized in a cold biocidal chemical solution (J.B. Dental, Irving, TX) for a minimum of 12 h at room temperature.

Table 1 Alloy compositions in weight percent [19].

	Ni	Cr	Mo	Fe	Al	Other[a]
Neptune	63.36	20.95	8.40	1.73	0.16	4.10 Nb, Si, Mn, Ti
Vera Bond	77.36	12.27	4.84	0.14	2.76	1.67 Be, Co, Ti, Si

[a] Less than 1.0% unless otherwise indicated.