Journal of Dentistry 66 (2017) 91–101

Contents lists available at ScienceDirect

Journal of Dentistry

journal homepage: www.elsevier.com/locate/jdent

Full length article

The effect of metal ions released from different dental implant-abutment couples on osteoblast function and secretion of bone resorbing mediators☆

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ARTICLE INFO

Keywords:
Dental implants
Peri-implant bone loss
Platform-switching
Titanium
Corrosion
Ion release

ABSTRACT

Objectives: The etiology of the reduced marginal bone loss observed around platform-switched implant-abutment connections is not clear but could be related to the release of variable amounts of corrosion products. The present study evaluated the effect of different concentrations of metal ions released from different implant abutment couples on osteoblastic cell viability, apoptosis and expression of genes related to bone resorption.

Methods: Osteoblastic cells were exposed to five conditions of culture media prepared containing metal ions (titanium, aluminum, vanadium, cobalt, chromium and molybdenum) in different concentrations representing the amounts released from platform-matched and platform-switched implant-abutment couples as a result of an earlier accelerated corrosion experiment. Cell viability was evaluated over 21 days using the Alamar Blue assay. Induction of apoptosis was measured after 24 h of exposure using flow cytometry. Expression of interleukin-6, interleukin-8, cyclooxygenase-2, caspase-8, osteoprotegerin and receptor activator of nuclear factor kappa-B ligand (RANKL) by osteoblastic cells were analysed after exposure for 1, 3 and 21 days using real-time quantitative polymerase chain reaction assay

Results: Metal ions in concentrations representing the platform-matched groups led to a reduction in cell viability ($P < 0.01$) up to 7 days of exposure. Stimulated cells showed higher rates of early apoptosis ($P < 0.01$) compared to non-treated cells. Metal ions up-regulated the expression of interleukin-6, interleukin-8, cyclooxygenase-2 and RANKL in a dose dependent manner after 1 day of exposure ($P < 0.05$). The up-regulation was more pronounced in the groups containing the corrosion products of platform-matched implant-abutment couples.

Conclusion: Osteoblastic cell viability, apoptosis, and regulation of bone resorbing mediators were significantly altered in the presence of metal ions. The change in cytokine levels expressed was directly proportional to the metal ion concentration.

Clinical significance: The observed biological responses to decreased amounts of metal ions released from platform-switched implant-abutment couples compared to platform-matched couples may partly explain the positive radiographic findings in respect to crestal bone level when utilising the "platform-switching" concept, which highlights the possible role of corrosion products in the mediation of crestal bone loss around dental implants

1. Introduction

Dental implants have been widely used for the replacement of missing teeth in fully and partially edentulous patients. According to the American Academy of Implant Dentistry, 3 million people in the United States have dental implants and that number is growing by 500,000 a year [1]. The use of endosseous dental implants was initiated by the discovery that these implants could be anchored in the jawbone with direct bone contact [2,3]. In 1991, Zarb and Albrektsson described the osseointegration phenomena as "a process in which a clinically asymptomatic rigid fixation of alloplastic material is achieved and maintained in bone during functional loading" [4] For proper osseointegration, several factors must be controlled [5,6], including biocompatibility of the implant material, design and surface of the implant, the condition of the tissues in the implant site, the surgical techniques, and loading procedures [5]. Biocompatibility of an implant

☆ Work submitted in partial fulfillment of the PhD degree of GOA.

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http://dx.doi.org/10.1016/j.jdent.2017.08.002
Received 13 March 2017; Received in revised form 30 June 2017; Accepted 2 August 2017

material is closely related to its susceptibility to corrosion [7]. Therefore, titanium (Ti) has been the material of choice for dental implants due to its superior corrosion resistance behaviour and desirable mechanical properties [8,9].

An important parameter in the long-term success of dental implants is the stability of the peri-implant bone. Previous literature has showed that alterations in the connection geometry of the dental implant-abutment interface, such as platform-switching, may lead to a decrease in peri-implant bone loss that occurs through time [10,11]. Platform-switching is defined as a protocol that includes smaller diameter restorative components that have been placed onto larger diameter implant restorative platforms – the outer edge of the implant-abutment interface is horizontally repositioned inwardly and away from the outer edge of the implant platform [10]. Nevertheless, the etiology for this difference is still questioned. A recent study [12] demonstrated an increase in the amount of metal ions released through accelerated corrosion from platform-matched compared to platform-switched implant-abutment couples.

The role of implant corrosion products in peri-prosthetic osteolysis has been extensively demonstrated in the orthopedic literature [13,14]. This phenomenon may occur as corrosion and wear products can influence the metabolic pathways of various cells including macrophages, lymphocytes, fibroblasts, osteoclasts, and osteoblasts [13,14]. Osteoblasts exposed to cobalt (Co) and chromium (Cr) ions undergo a dose dependent reduction in proliferation [15]. Titanium (Ti) ions at concentrations of 10 ppm or higher for 24 h were found to be toxic [16]. Additional past studies have demonstrated that nontoxic concentrations of metal ions influence the differentiation and function of osteoblastic cells *in vitro* [17,18].

Metal ions/particles may also stimulate osteoblasts to produce pro-inflammatory mediators that contribute to the overall inflammatory process involved in peri-prosthetic osteolysis [13,19–25]. It has been shown that cobalt ions stimulate increased prostaglandin E2 (PGE2) secretion in primary human osteoblasts [26]. This was preceded by up-regulated cyclooxygenase COX-1 and COX-2 gene expression [19,26,27]. Secretion of interleukins 6 and 8 (IL-6 and IL-8) by osteoblasts in response to Ti and other experimentally derived wear particles/ions has also been previously reported [28–30]. Receptor activator of nuclear factor kappa-B ligand (RANKL) is another important protein in peri-prosthetic osteolysis and acts by stimulating osteoclastogenesis [19]. Osteoprotegerin (OPG) is an inhibitor of RANKL. Mine et al. revealed that Ti ions enhanced the expression of RANKL in osteoblast-like cells, suggesting that Ti ions may have adverse effects on bone remodelling at the interface of dental implants and tissues [31].

Although several investigations have documented the potential toxicity and the ability of metal ions/particles to stimulate cytokine production in cultured cell systems [32–34], little is known regarding cell apoptosis, or programmed cell death [35]. It has been suggested that biocompatibility testing should include assessment of apoptosis [36] which is featured by the stimulation of cysteine proteases called caspases. An *in vitro* study [37] showed that Ti particles could induce apoptosis in osteoblasts which may lead to suppressed bone formation.

Although the orthopedic literature is replete with studies regarding the influence of corrosion products on the peri-prostheic tissues and cells [13–37], the dental literature, however, contains little information about the direct interaction between metal ions released from dental implants and osteoblasts from peri-implant tissues. This interaction may provide important insights into the pathogenesis of the observed marginal bone resorption around dental implants [38,39]. In highly corrosive environments, such as the oral cavity, metals, including those of the implant and abutment materials, are prone to degradation [40,41]. The combination of an acidic medium, due to inflammation, presence of acidogenic bacteria, fluorides or food intake, and the micromotion, resulting from occlusal forces, can lead to disruption of the oxide layer protecting the titanium surface [12,40–43]. A recent study [12] demonstrated that Ti implants connected to platform-matched abutments released significantly larger amounts of corrosion elements compared to implants connected to platform-switched abutments, following an accelerated corrosion process. The authors' hypothesis was that this difference in corrosion may be significant on a cell metabolic level in order to change the peri-implant bone homeostasis [12].

The aim of the present study was to investigate the effect of such differences in metal ion concentrations on cell viability, apoptosis, and inflammatory gene expression of human osteoblastic cells cultured within conditioned culture media containing the different concentrations of metal ions obtained from the earlier study [12]. The null hypothesis was that there would be no difference between groups regarding the aforementioned variables.

2. Materials and methods

2.1. Preparation of culture media containing metal ions

Five different conditions of culture media solutions were prepared containing different levels of metal ions obtained from the respective 5 groups of a recent study [12] that evaluated the levels of metal ions released from different implant abutment couples as a result of accelerated corrosion [44]. The metal ions corresponded to the following groups [12]: implants connected to platform-matched titanium (Ti6Al4V) abutments (TM), implants connected to platform-switched titanium (Ti6Al4V) abutments (TSW), implants connected to platform-matched cobalt-chrome (CoCr) abutments (CM), implants connected to platform-switched cobalt-chrome abutments (CSW) and unconnected titanium implants (UI). The amount of mismatch was 0.5 mm between the platform-switched and platform-matched abutments [12]. The concentrations of the measured elements [12] which were used in this study are presented in Table 1. To prepare culture medium containing these concentrations, single element standard solutions for ICP-MS for each measured element (titanium (Ti), vanadium (V), aluminum (Al), cobalt (Co), chromium (Cr) and molybdenum (Mo)) were utilized (TraceCERT®, Sigma-Aldrich Company Ltd.,Dorset, England). Each single standard solution of each element was sterilized by passing through 0.22 μm membrane filters (Millex, Merck Millipore Ltd., Germany) before diluting in culture medium (Clonetics™ OGM™ BulletKit™, Lonza, Walkersville, MD, USA). To reach the desired concentrations of the test solutions, the single element standard solutions were diluted with the serum-added culture medium, under pH monitoring, according to the method described by Taira et al. [45]. No visual precipitation was formed after adding the standard elements and the pH of the prepared solutions was measured immediately after preparation. Metal ion-free culture medium was used as a reference solution (REF) and served as the control group.

Table 1
Levels of metal ions (ppb) present in treated culture media solutions[12].

Test Groups	Code	Levels of Metal Ions (ppb)						
		Ti	Al	V	Co	Cr	Mo	Total
Unconnected implant	UI	998						998
Connected platform matched titanium alloy abutment (6 mm)	TM	1250	67	60				1377
Connected platform switched titanium alloy abutment (5 mm)	TSW	1080	57	36				1137
Connected platform matched cobalt-chrome abutment (6 mm)	CM	678			219	27	10	934
Connected platform switched cobalt-chrome abutment (5 mm)	CSW	623			122	11	6	762