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Cytotoxicity and terminal differentiation of human oral keratinocyte by indium ions from a silver–palladium–gold–indium dental alloy

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ARTICLE INFO

Article history:
Received 11 April 2014
Received in revised form
13 August 2014
Accepted 10 November 2014

Keywords:
Silver–palladium–gold–indium dental alloy
Corrosion resistance
Indium ions
Cytotoxicity
Terminal differentiation
Human oral keratinocytes

ABSTRACT

Objective. Dental alloys containing indium (In) have been used in dental restoration for two decades; however, no study has investigated the biological effects of In ions, which may be released in the oral cavity, on human oral keratinocytes. The objective of the present study was to investigate the biological effects of In ions on human oral keratinocyte after confirming their release from a silver–palladium–gold–indium (Ag–Pd–Au–In) dental alloy.

Methods. As a corrosion assay, a static immersion tests were performed by detecting the released ions in the corrosion solution from the Ag–Pd–Au–In dental alloy using inductively coupled plasma atomic emission spectroscopy. The cytotoxicity and biological effects of In ions were then studied with In compounds in three human oral keratinocyte cell lines: immortalized human oral keratinocyte (IHOK), HSC-2, and SCC-15.

Results. Higher concentrations of In and Cu ions were detected in Ag–Pd–Au–In ($P < 0.05$) than in Ag–Pd–Au, and AgCl deposition occurred on the surface of Ag–Pd–Au–In after a 7-day corrosion test due to its low corrosion resistance. At high concentrations, In ions induced cytotoxicity; however, at low concentrations (~0.8 In^{3+} mM), terminal differentiation was observed in human oral keratinocytes. Intracellular ROS was revealed to be a key component of In-induced terminal differentiation.

Significance. In ions were released from dental alloys containing In, and high concentrations of In ions resulted in cytotoxicity, whereas low concentrations induced the terminal differentiation of human oral keratinocytes via increased intracellular ROS. Therefore, dental alloys containing In must be biologically evaluated for their safe use.

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http://dx.doi.org/10.1016/j.dental.2014.11.006
0109-5641/

1. Introduction

High-carat gold (Au) alloys have been used for centuries in dental restorations; however, the market price of Au has rapidly increased with continued use [1,2]. The traditional high-carat Au alloys have been substituted with low-carat Au alloys, such as the Ag–Cu–Au alloy [3]. Since a gold-yellow color dental alloy with two white colored metals, i.e., palladium (Pd) and indium (In), was invented two decades ago, the silver–palladium–gold–indium (Ag–Pd–Au–In) dental alloy has been widely used in dental restoration. Three primary reasons for the use of this alloy are its (1) gold-yellow color, (2) increased bonding strength with porcelain, and (3) increased tarnish and corrosion resistance when containing 5–10% In [4,5]. However, the corrosion resistance of dental alloys containing In depends on the proportion of each element; thus, In ions could potentially be released into the oral cavity from the corroded alloy, and this potential release of In ions has been underestimated to date [6].

The embryotoxicity and teratogenicity of In ions has been reported in the literature [7,8]. In addition, In itself exhibits cytotoxicity in human leukocytes and lymphocytes [9]. Nevertheless, In has been used as a supplemental element in gallium-based liquid alloys instead of dental amalgam due to the relative biocompatibility with L929 (mouse fibroblasts) for <1 mM of In ions [10,11]. A biological study of extracts from dental alloy containing In was performed with Balb/c 3T3 (mouse embryos) and exhibited toxicity; in addition, the released In ions concentration was determined to be approximately 0.2 mM for 72 h of incubation in tissue culture media [12,13]. However, cytotoxicity tests of In ions with human oral keratinocytes have not been performed despite the use of dental alloys containing In for dental restoration. Previous studies have demonstrated that the cytotoxicity results could differ dramatically based on the cell lines employed, especially those originating from different types of tissue [14,15]. Therefore, cytotoxicity studies of dental materials with human oral keratinocytes have been widely performed because extracts or ions released from dental materials anatomically encounter oral keratinocytes of epithelium and thus affect the biocompatibility of the oral epithelium [16–18]. Hence, this study considers In-induced cytotoxicity with human oral keratinocytes.

Aside from cytotoxicity, other biological effects of metal ions have been studied in the use of dental alloys in dental restoration. In clinical situations, the released metal ions might be diluted to non-cytotoxic levels in the oral cavity by saliva. Hence, the biological effect of metal ions under non-cytotoxic levels must be investigated such that these ions can be safely used as components of dental alloys [19–21]. Generally, a high concentration of metal ions induces cytotoxicity; however, other biological results are observed at lower concentrations [22,23]. The metal ions Au^{3+}, Ag^{+}, Pd^{2+}, Cu^{2+}, Ni^{2+}, and In^{3+} resulted in no cytotoxicity at concentrations of up to 5 mM [19]. A few millimoles of Ni^{2+} or Cu^{2+}, which is under the cytotoxic concentration, have been demonstrated to affect epithelial–mesenchymal transition (EMT) and fibrosis in human skin keratinocytes, which are considered to be associated with cancerization [20,21]. Therefore, the biological effects of In at non-cytotoxicity concentrations should be evaluated for the use of In in dental alloys.

The biological effects of metal ions on oral keratinocytes under cytotoxic concentrations can be divided into two different types: cancerization and terminal differentiation of keratinocytes. EMT is used as a representative event to evaluate cancerization. During EMT, fibronectin is up-regulated, and over-expression of epidermal growth factor receptor (EGFR) phosphorylation is detected [20,24]. Fibronectin is a mesenchymal marker that is highly expressed in mesenchymal cells and is up-regulated in cancerization [25]. EGFR is the transmembrane receptor in keratinocytes for uptaking various growth factors (i.e., epithermal growth factor, tissue growth factor-alpha) and plays a pivotal role in regulating keratinocyte proliferation, differentiation, and transformation [26]. The over-expression of EGFR was detected in oral keratinocyte cancerization [27]. However, the terminal differentiation of oral keratinocyte, which plays a pivotal role in maintaining the physical barrier of oral mucosa, could be induced. During terminal differentiation, keratinocytes produce increasingly more keratin and involucrin, which contributes to the physical barrier and formation of a cell envelop to protect the outermost part of the oral epithelium [24].

Biological events induced by metal ions have been determined to be related to an increased concentration of intracellular reactive oxygen species (ROS) because these species serve as signaling intermediates in cellular signaling pathways, including cell differentiation, proliferation and apoptosis [20,28]. Previous studies suggested that increased intracellular ROS may play an early causal role in the terminal differentiation in keratinocytes [29,30]. Therefore, the terminal differentiation of oral keratinocytes via increased intracellular ROS might be considered a potential biological event [31,32].

Thus, the objective of the present study was to investigate the biological effects of In ions on human oral keratinocytes after confirming the release of In ions from a Ag–Pd–Au–In dental alloy. The null hypothesis of this study was that In ions do not induce significant biological effects on human oral keratinocytes.

2. Materials and methods

2.1. *Dental alloys*

Two dental alloy compositions, Ag–Pd–Au (70% Ag–15% Pd–5% Au) and Ag–Pd–Au–In (60% Ag–15% Pd–10% In–5% Au), were selected for investigation after a preliminary study, and rectangular-shaped specimens were prepared using a conventional metal alloy casting procedure. To precisely control the composition and compare the characteristics of the alloys, we fabricated our own specimens rather than purchasing commercial alloys. Briefly, each alloy was prepared by melting Ag, Pd, Au, and/or In pellets (purity 99.99%, LS nikko, Ulsan, Korea). After weighing each component for the designated composition, three samples of 15 g of the mixture were melted three times in an arc melter to promote chemical homogeneity. The chamber was evacuated to 5×10^{-3} Torr, and high-purity argon gas was introduced until the pressure reached 200 torr before