Biocompatibility of gold and stainless steel chains used for forced eruption of impacted teeth – an in vitro investigation

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Objective. Surgical approaches for the mobilization of impacted teeth involve the use of gold chains to connect the impacted tooth with the orthodontic appliance. In this study we have compared the local effects gold plated stainless steel with stainless steel specimen using an in vitro model of the gingival mucosa and monolayer cultures of cells of the alveolus.

Study Design. Local effects on differentiation, proliferation, and apoptosis and inflammatory response were tested using organotypic cultures of gingival cells. Cytotoxicity was measured by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assays with monolayer cultures of human periodontal cells.

Results. The data obtained in this study could not reveal any differences in favor of using gold plated chains during the mobilization of impacted teeth.

Conclusion. For patients not suffering from allergies against nickel there might be no rationale to favor gold plated chains, as there are no functional aspects to favor gold plated chains over stainless steel chains. (Oral Surg Oral Med Oral Pathol Oral Radiol 2013;116:159-168)

Treatment of impacted teeth can be undertaken using a variety of surgical and orthodontic approaches, including open and closed eruption techniques. Open exposure may result in significant soft and hard tissue loss and the 'closed eruption technique',[1-3] utilizing a surgically repositioned flap, is often the preferred technique for deeply impacted teeth. For alignment metal chains are commonly used to connect the impacted tooth to an orthodontic appliance.[1,4-6] The chains used can be uncoated stainless steel or gold plated steel chains.

Recent evidence implicates gold as an allergen, causing local and systemic effects.[7,8] Patients with dental restorations containing gold are prone to develop contact allergies to the material.[9,10] In the Swedish population gold is the second most common contact allergen after nickel.[11] In the U.S. gold ranks among the top 10 allergens (North American Dermatitis Group) and similar figures apply for Europe.[12]

Stainless steel used in orthodontic materials may initiate a local response that could include cytotoxicity and genotoxicity.[13-15] Tissue reactions dependent on the corrosion potential and quality and quantity of released elements. Copper, zinc, nickel and silver all have a significant impact on cell viabilities and nickel is the most relevant contact allergen worldwide.[16]

Uncoated stainless steel chain and gold plated chain are used for connecting impacted teeth to orthodontic devices and may cause inflammatory responses or even cytotoxicity and the cells affected include keratinocytes, fibroblasts and osteoblasts. However, to the best of our knowledge, no clinical or experimental studies have been undertaken to investigate local cellular responses to either material; the preference of using a gold-plated or uncoated stainless steel chain appears without clinical or biological evidence.

The aim of this study was to compare cellular effects of gold plated stainless steel with stainless steel on gingival mucosa using an in vitro model. The effects of the 2 metals on cell differentiation, proliferation, inflammatory cytokine synthesis, and apoptosis were evaluated and compared.

MATERIALS AND METHODS

Cell culture

Gingival tissue and alveolar bone was obtained from patients following extraction of third molars. Informed

Statement of Clinical Relevance

Surgical approaches for the mobilization of impacted teeth involve the use of gold chain. Their use appears without biological evidence. The data obtained in this study could not reveal any differences in favor of using gold plated chains.

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Received for publication Dec 20, 2012; returned for revision Feb 6, 2013; accepted for publication Feb 19, 2013.

2212-4403/$ - see front matter
http://dx.doi.org/10.1016/j.oooo.2013.02.015

consent was obtained from patients who had to undergo removal of wisdom teeth for medical reasons. The local Ethics Committee (Medical Faculty, University of Heidelberg; Votes 80/94 and S147/2010) approval was obtained.

The isolation of gingival cells was performed as described before.[17] Briefly, gingival tissues were separated in epithelial and connective tissue. Epithelial tissue was dissociated using dispase (2.4 U/mL, 37°C, 30 min). Cells were seeded and subcultured in keratinocyte growth medium (PromoCell, Heidelberg, Germany) at 37°C in a humidified 5% CO_2 incubator (Thermo Scientific, Langenselbold, Germany). Keratinocytes were immortalized by the E6 and E7 genes of human papilloma virus 16 (HPV-16) using recombinant retroviruses.[18]

Gingival fibroblasts were established from explant culture from the remaining connective tissue as described by Tomakidi et al.[17] Cultures of osteoblasts of the alveolar bone were established as described by Diercke et al.[19]

Gingival fibroblasts osteoblasts and L929 murine fibroblasts were cultured in Dulbecco's modified eagle medium (DMEM) (Invitrogen, Karlsruhe, Germany) supplemented with 10% fetal calf serum (FCS), 2 mM L-glutamine, antibiotics and antimyotics at 37°C in a humidified 5% CO_2 incubator. Immortalized gingival keratinocytes were used between passages 104 and 106. Gingival fibroblasts were used between passages 4 and 6. Osteoblasts were use in passage 6.

Organotypic cultures of human gingival cells were established as described by Tomakidi et al.[17]: bovine collagen type I (Life Technologies, Darmstadt, Germany) was polymerized at 3 mg/mL in Hanks' buffered solution (HBS, 10×), gingival fibroblasts were added in FCS at a concentration of 1×10^5 cells/mL collagen and collagen gels were polymerized by neutralization with NaOH (1 N). Collagen gels containing gingival fibroblasts were allowed to polymerize and were immersed in organotypic culture medium [DMEM/Ham's F-12 (1:1 Mixture, Life Technologies, Darmstadt, Germany)] supplemented with bovine pituitary extract [0.004 mL/mL], epidermal growth factor (recombinant human) [0.125 ng/mL], insulin (recombinant human) [5 µg/mL], hydrocortisone [0.33 µg/mL], epinephrine [0.39 µg/mL], transferrin, holo (human) [10 µg/mL], $CaCl_2$ [0.06 mM]. After 24 h gingival keratinocytes (7×10^5 cells per collagen-gel) were seeded on the surface of the collagen gels and allowed to adhere for 24 h. At this stage organotypic cultures were lifted onto metal grids allowing air contact of the upper keratinocyte layer. Organotypic culture medium was changed every second day and the cultures were matured for 21 days.

Preparation of gold plated stainless steel specimen and stainless steel discs and confrontation with organotypic cultures of human gingival cells

Discs were manufactured from 1.4305 (X 8 CrNiS 18 9) stainless steel (C: 0.06%, Si: 0.356%, Mn: 1872%, P: 0.029%, S: 0.277%, Cr: 17.732%, Ni: 8678%, N: 0.034%, Cu: 0.389%).

Discs were gold electroplated; the plating thickness amounting to 5-20 µm.

Mature organotypic cultures days were confronted with gold-plated stainless steel and stainless steel discs by direct application of 5×1 mm specimen on the epithelial layer of the organotypic cultures. The discs were thoroughly rinsed in distilled water, cleaned in an ultrasonic bath for 15 min at 30°C, rinsed in distilled water and sterilized by autoclaving. Confrontation was for 24 h. The control organotypic cultures remained untreated.

For the assessment of differentiation, proliferation and apoptosis by immunofluorescent staining 5 organotypic cultures per group were evaluated. The organotypic cultures were carefully frozen over liquid nitrogen and embedded in Tissue-Tek (Sakura Finetek, Staufen, Germany). Cryosections (10 µm) were mounted on adhesive slides, air-dried and fixed with methanol/acetone (1:1) for 10 min at −20°C and frozen at −80°C until analyzed.

Quantification of pro-inflammatory cytokines was performed for additional 5 organotypic cultures per group. Supernatants were harvested and snap frozen in liquid nitrogen and stored at −80°C until analysis.

Immunofluorescent staining

Incubation with primary antibodies (anti-ck14, clone LL002, 1:50; anti-involucrin, clone SY5 1:50, anti-filaggrin, rabbit polyclonal, 1:100 all from Abcam, Cambridge, UK, Ki-67, clone MIB-1, DAKO, Hamburg, Germany; cleaved caspase-3 (Asp175) (5A1E) 1:600, Cell signaling Technologies/New England Biolabs, Frankfurt, Germany) was performed overnight at 4°C. After washing (3×10 min in PBS) sections were incubated with fluorochrome-conjugated antibodies (Jackson-ImmunoResearch/Dianova, Hamburg, Germany), for 1 h at room temperature. Sections were mounted in antifade reagent with DAPI (4′, 6-diamidino-2-phenylindole) as a counterstain.

Microphotographs were taken using a Leica DMRE microscope equipped with a digital camera (DFC300 FX, Leica, Bensheim, Germany). Image acquisition and processing was done using Leica application suite software (Leica, Bensheim, Germany). Proliferation and apoptotic indexes were assessed by determining the total number of cells on the basis of the nuclear DAPI stain and the number of cells stained positively for Ki-67 or cleaved