Biocompatibility of Temporary Anchorage Devices Using an in vitro Cell Culture Model

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Abstract

Objective: The objective of this study was to evaluate the biocompatibility of five commercially available orthodontic miniscrews/temporary anchorage devices.

Materials and Methods: A cell culture system using commercially available human gingival fibroblasts was utilized. Miniscrews/temporary anchorage devices obtained from Ormco, Ortho Organizer, 3M Unitek, American Orthodontics, and Rocky Mountain Orthodontics were immersed in culture medium for either 48 hours or twoweeks. The surface area to volume ratio of the eluates produced by this method was within the suggested ISO range. Gingival fibroblasts were exposed to miniscREW/temporary anchorage device conditioned culture medium for 24 hours. Biocompatibility and cytotoxicity were measured using colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and lactate dehydrogenase (LDH) assays. Results were analyzed using ANOVA with Tukey’s post-test and $P \leq 0.05$.

Results: LDH analysis showed no differences in disruption of cell membrane integrity, suggesting that the miniscrew/temporary anchorage devices were not cytotoxic to gingival fibroblasts ($P > 0.05$ for all comparisons). MTT analysis, used to quantify viable cells, demonstrated that 48 hour conditioned medium significantly affected cell viability ($P < 0.001$ for all treatments compared to control). In contrast two week conditioned medium from all five devices did not affect cell viability compared to control.

Conclusion: The results of this study suggest that miniscrews/temporary anchorage devices demonstrate longer-term biocompatibility with gingival fibroblasts and that the composition of the devices is not cytotoxic to oral cells. The general lack of data regarding the cytotoxicity of miniscrews/temporary anchorage devices in orthodontic treatment warrants further investigation.

Keywords: Biocompatibility; Cytotoxicity; Fibroblasts; Miniscrews; Temporary Anchorage Devices

Introduction

Orthodontic anchorage, defined as a resistance to unwanted tooth movement, has been a long-standing major consideration in orthodontics since the introduction of fixed appliances [1,2]. Typically to achieve orthodontic movement of a single tooth, the anchorage may be provided by a group of teeth whereby the total root surface area of the anchor unit will be greater than the root surface area of the single to be moved tooth [3]. For every movement of a tooth in the desired direction, an equal and opposite force is distributed to the anchoring teeth, creating the potential for unwanted tooth movement. For example, when a canine tooth is retracted using a single molar tooth as a source of anchorage, the molar will move mesially to some extent as the canine moves distally.

Absolute anchorage, a method used to avoid unwanted tooth movement in orthodontics, can be achieved by appliances such as mini-implants, miniscrews, or temporary anchorage devices (TADs) [4]. Indeed mini-screws in orthodontics have become the most commonly used skeletal temporary anchorage device [5]. Anchorage is defined as no movement of the anchorage unit as a consequence to the reaction forces applied to move teeth [3]. In current orthodontic practice, TADs can be used for a variety of movements including mesial and distal movements, anterior retraction and protraction, intrusion, extrusion, uprighting, corrections of midlines, transversal problems, occlusal cant, and open bite or deep bite [6-9]. For example, the devices have been successfully used as absolute anchorage to reduce overbite in non-growing patients [10]. An advantage of anchorage devices is that they can be incorporated into orthodontic treatment without the necessity of patient compliance [11-13]. In a survey of Australian orthodontists, 77% of those responding use TADs [14].

Although orthodontic miniscrews/TADs are widely used [15,16] data concerning the biocompatibility of these devices in human, animal, or cell culture models is very limited [17]. Materials placed in the oral cavity must be non-toxic to cells, must not illicit inflammatory responses, and must not be subject to corrosion or breakdown leading to the dissolution of metal or other ions into tissues and oral fluid [18]. Malkoc et al. [6] evaluated the cytotoxic effects of orthodontic mini-implants on human gingival fibroblasts and mouse osteoblasts in culture. Five different TADs were analyzed: the Orthodontic Mini Implant, MTN, AbsobAnchor, IMTEC Ortho, and VectorTAS. The authors reported no significant difference in cell indices between control and treatment groups in the gingival fibroblast model.

The objective of this study was to provide more evidence for the biocompatibility of TADs by evaluating the cytotoxicity of five commercially available devices used in humans and oral fluid. The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is widely used for determining the cytotoxic potential of compounds, including dental materials[19]. Additional evidence for cytotoxicity was provided in the current study by using a colorimetric lactate dehydrogenase (LDH) assay.

Materials and Methods

Preparation of Eluates from Miniscrews

Miniscrews/TADs were obtained from five companies for this study: 1) Vector TAS mini-screws composed of Ti6Al4V (Ormco Corporation, Orange, CA, USA), 2) Anchor Pro temporary orthodontic anchorage mini-screws composed of Grade 5 titanium (OrthoOrganizers, Carlsbad, CA, USA), 3) Unitek temporary anchorage devices composed of Grade 23 titanium alloy (American Orthodontics, Sheboygan, WS, USA), and 4) Aarhus System mini-screws composed of Ti6Al4V high strength titanium alloy (American Orthodontics, Denver, CO, USA), and 5) RMO Dual Top temporary anchorage devices composed of surgical grade titanium alloy (Rocky Mountain Orthodontics, Denver, CO, USA).
The devices as received from the vendor were sterilized by ultraviolet light for 24 hours without cleaning or wiping the surface. Two devices of each type were incubated in 5 ml serum-free culture medium at 37 °C in 5% carbon dioxide for 48 hours. The culture medium was collected for analysis after 24 hours before 5 ml of fresh medium was added. The devices were then incubated for an additional 2 weeks. Culture medium conditioned by the devices for 48 hours or 2 weeks was sterile-filtered through a 0.22µm filter before application to cultured cells.

**Biocompatibility/Cytotoxicity Assays**

Human gingival fibroblasts (ScienCell, Carlsbad, CA, USA) between passages 2-5 were seeded into wells of a 24 well tissue culture plate. At approximately 100% confluence (no plastic substrate could be seen between adherent cells) the fibroblasts were treated with the culture medium conditioned by the miniscrews/TADS (n = 5 for each treatment) for 24 hours at 37 °C in 95% air and 5% carbon dioxide. Cells were incubated in 0.002% chlorhexidine as the positive control because we have shown in other studies that chlorhexidine (CHX) is cytotoxic to fibroblasts with the MTT assay [20]. Cells were incubated in serum free medium only as the negative control as used as the measure of cell survival.

After the 24 hour treatment, an MTT assay was performed on the fibroblasts according to the protocol of the Sigma Cell Growth Determination Kit MTT Based (Sigma Aldrich Chemical Co., St. Louis, MO, USA). Fibroblasts were incubated with the MTT reagent for 3 hours. The resulting formazan crystals were dissolved with the MTT solubilization solution, and the optical density of samples (n = 15 for each treatment or controls) was determined at 570 nm using a SpectraMax M3 (Molecular Devices, Sunnyvale, CA, USA) microplate reader. For MTT assays data were reported as relative percent cell viability, setting the negative control to 100%.

Prior to initiating the MTT assay, 50 microliters of the culture medium (n = 15 for each treatment or controls) was placed into wells of a 96-well microtiter plate. 100 microliters of LDH assay mixture (In vitro Toxicology Assay Kit, Sigma-Aldrich) was added to each well followed by an incubation of 30 minutes at room temperature. The optical density of samples was determined at 490 nm. Absorbance values for LDH assays were corrected for total cell number by fixing and staining adherent cells with crystal violet blue. Stained cells were eluted with 1% SDS solution, and absorbance was read at 595 nm. Corrections were calculated by dividing the OD 490 nm for a given well (LDH value) by the OD 595 nm reading of the same well (cell number). For LDH assays data are reported as corrected absorbance values, with comparatively higher values indicating a higher degree of cell disruption.

Statistical analyses were completed using one-way analysis of variance (ANOVA) with Tukey’s ad hoc multiple comparison post-test. A value of P ≤ 0.05 was considered to be statistically significant.

**Results**

The International Organization for Standardization (ISO) 10993 set is a series of standards for the evaluation of medical devices. ISO standards recommend that solid devices be tested for biocompatibility within the range of 0.5 to 6.0 cm²/ml [21]. Because of the complex structure of the device it was difficult to calculate the exact surface area to volume ratio for the miniscrews/TADs. The dimensions of the Vector-TAS miniscrew analyzed in this study were 8 mm in length by 1.4 mm in diameter. Using the formula to calculate the surface area of a cylinder which is 2πr² + 2πrh, the surface area determined to be 3.8 cm². This is in close agreement to 3.9 cm² for the Vector-TAS miniscrew reported by Al Samakeh et al [22]. This miniscrew was thus tested in this study at a surface area to volume ratio of 0.78 cm²/ml which is within the ISO recommended range.

The LDH assay can be used to determine disruptions in cell membrane integrity and to assess relative amounts of dead cells in a culture. Culture medium conditioned by the miniscrews/TADs for either 48 hours (Figure 1) or 2 weeks (Figure 2) showed no deleterious effects on fibroblast cell membrane integrity. Absorbance values did not differ from negative controls, suggesting that none of the five devices tested were cytotoxic to the gingival fibroblasts (P > 0.05 for all comparisons to control and between devices).

The MTT assay detects living cells only, which allows for a measurement of cytotoxicity, proliferation, or activation [23]. The MTT assay is used to quantify viable cells based on mitochondrial
viability. When fibroblasts were treated with medium conditioned by the miniscrews/TADs for 48 hours (Figure 3), cell viability was significantly decreased by all devices compared to control (P < 0.001 for all treatments to control). In contrast, medium conditioned by the miniscrews/TADs for two weeks had no significant effect on fibroblast viability (Figure 4).

Discussion

The stability and biocompatibility of orthodontic miniscrews/TADs is vital to the clinical success of the product [24,25]. Although the devices have a common composition of titanium few published reports analyze the cytotoxic potential of such devices. To our knowledge based on a search of the literature, only two studies have been published to directly analyze the cytotoxicity potential of commercial TADs. Culture medium was conditioned by the indicated device for 2 weeks and then used to treat human gingival fibroblasts for 24 hours prior to MTT assay. MTT assay results are reported as % cell survival compared to negative control arbitrarily set to 100%. Cell survival was not affected in the presence of TAD conditioned medium (P < 0.001) and in the presence of chlorhexidine (CHX) used as a positive control. TAS: Vector TAS, AP: AnchorPro, UT: Unitek, AS: Aarhus System, DT: Dual Top.

Conclusions

The main aim of this study was to evaluate the biocompatibility of five commercially available orthodontic miniscrews/TADs. No sustained deleterious effects of materials solubilized from the devices were observed in cultured cells. Orthodontic miniscrews and temporary anchorage devices are biocompatible and demonstrate no long-term cytotoxic effects on cells of the oral cavity.

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References

4. Antoszewska J, Rątowicz-Wójcik K, Kawała B, Matthews-Brzozowska T. Biological factors involved in implant-anchored orthodontics and in response to eluates produced by miniscrews/TADs [26]. While this is an interesting supposition, fibroblasts currently are the standard in vitro cell model for toxicity determinations [27].

Cytotoxic characteristics were observed in the 48 hour eluates of all five miniscrews/TADs but this was not corroborated by LDH assay. In HepG2 cells the MTT assay revealed toxicity before any effect with the LDH leakage assay or a neutral red assay [28]. It is plausible that the MTT assay demonstrates a higher level of sensitivity in the cell culture system used for this study, and detected cytotoxicity that was below the level of detection of the LDH assay.

There were no observed cytotoxic effects of the 2 week eluates from any of the five miniscrews/TADs tested. We surmise that any coating materials on the new devices as packaged by the vendors was washed off or otherwise removed during the initial 48 hour incubation. A statistically significant decrease in cell viability after exposure to miniscrews/TADs incubated in saline at pH 4.0 has been reported [26]. It is possible that initial solubilization of materials on the surface of new miniscrews/TADs creates a lower pH environment in the conditioned medium that is detrimental to cell viability. Alternately there may be some dissolution of metal or other ions from the surfaces of the devices when they are first exposed to the culture medium. In the oral cavity, this might result in a brief time of non-compatibility but it is likely that saliva would dilute the solubilized materials to non-toxic levels. After this initial period the data suggests that the temporary anchorage devices provide no detrimental effects to oral cavity tissues.

In summary the results shown here suggest that miniscrews/TADs examined in this in vitro study are biocompatible with gingival fibroblasts. The lack of data and the acceptance of miniscrews/TADs in orthodontic treatment warrants continued investigation of their biocompatibility as new products are introduced to the market.


