

Comparison of Biocompatibility of Different Materials of Mini-Implants: An *in Vitro* Study

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Abstract

Background: The objective of this study was to compare the biocompatibility of titanium-based and stainless steel-based orthodontic mini-screws/temporary anchorage devices (TADs). **Materials and Methods:** A cell culture system using commercially available human osteoblast cells was utilized. Mini screws/TADs obtained from Rivera Orthodontics were immersed in a culture medium for 24 h. The surface area to volume character of the eluates produced by this method was within the suggested ISO range. Human osteoblasts were exposed to a mini-screw/TAD conditioned culture medium. Biocompatibility and cytotoxicity were measured using a colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **Results:** MTT analysis, used to quantify viable cells, demonstrated that a 24-h conditioned medium significantly affected cell viability. **Conclusion:** When observed for 24 h, titanium-based implants showed less cell death than stainless steel implants. The long-term effect of implants on cell viability needs to be studied further.

Keywords: Clinical features, Gingival fibromatosis, Management, Pathogenesis.

INTRODUCTION

The field of orthodontics has witnessed remarkable advancements in recent years, with orthodontic mini-implants emerging as an essential tool for achieving stable anchorage during various treatment procedures. Anchorage has long been a challenge since the introduction of fixed appliances in orthodontics.^[1] Typically, the orthodontic movement of a tooth is anchored by a large group of teeth to minimize unwanted movements of the anchoring teeth. Adequate anchorage becomes difficult when posterior teeth are missing. Intraoral and extraoral auxiliary devices can be used to assist movement, but the effectiveness of these measures depends on the patient's cooperation.^[1] Mini-implants offer clinicians the ability to exert controlled forces on teeth, facilitating the movement of teeth with improved precision and efficacy. In 1997, Wehrbein *et al.*^[2] and Kanomi^[3] used mini-implants to anchor orthodontic movement. After these two studies, mini-implants were introduced as temporary skeletal anchorage devices and have been gaining popularity because their small size allows more placement sites in the oral cavity and reduces bone-healing time.^[4] The use of a stable point of anchorage has broadened the possibilities and predictability of orthodontic treatments that avoid tooth extraction and orthognathic surgery in patients with unfavorable bone support. This type

of anchorage has also been indicated in adult patients who would normally decline extraoral appliances, as well as in those having complete dentition lacking edentulous gaps.^[5-8] The material used to manufacture mini-implants must be non-toxic, biocompatible, and mechanically resistant to support tension and corrosion.^[8] Therefore, commercially pure titanium is the material of choice, as it is biocompatible and presents satisfactory mechanical properties.^[1]

Aims and objectives

The objective of this study was to provide more evidence for the biocompatibility of temporary anchorage devices (TADs) by evaluating the cytotoxicity of commercially available devices using cultured human osteoblasts and colorimetric assays for cell viability. The colorimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay is widely used for determining the cytotoxic potential of compounds.^[9]

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MATERIALS AND METHODS

Preparation of mini-screws

Mini-screws/TADs were obtained from Rivera company for this study:

1. 1.6×8 mm titanium-based implant
2. 2×14 mm stainless steel-based implant

The mini-implants as received from the vendor were sterilized by ultraviolet light for 24 h without cleaning or wiping the surface. The mini-implants were incubated in 5 mL serum-free culture medium at 37°C in 5% carbon dioxide for 48 h. The culture medium was collected for analysis after 24 h before 5 mL of fresh medium was added. The devices were then incubated. The culture medium was conditioned by the mini-implants for 48 h and was sterile-filtered through a 0.22 μ m filter before application to cultured cells.

Biocompatibility/cytotoxicity assays

Human osteoblasts were seeded into wells of a 24-well tissue culture plate. At approximately 100% confluence, the osteoblasts were treated with the culture medium conditioned by the mini-screws/TADs for 24 h at 37°C in 95% air and 5% carbon dioxide. Cells were incubated in serum-free medium only as the control and used as the measure of cell survival.

After the 24-h treatment, an MTT assay was performed on the osteoblasts according to the protocol of the Sigma Cell Growth Determination Kit MTT Based (Sigma Aldrich Chemical Co., St. Louis, MO, USA). Osteoblasts were incubated with the MTT reagent for 3 h. The resulting formazan crystals were dissolved with the MTT solubilization solution, and the optical density of samples was determined at 570 nm using a SpectraMax M3 (Molecular Devices, Sunnyvale, CA, USA) microplate reader. For MTT assays, data are reported as relative percent cell viability, setting the negative control to 100%. Before initiating the MTT assay, 50 microliters of the culture medium were placed into wells of a 96-well microtiter plate. The optical density of samples was determined at 490 nm.

RESULTS

The MTT assay detects living cells only, which allows for a measurement of cytotoxicity, proliferation, or activation.^[10] The MTT assay is used to quantify viable cells based on mitochondrial viability. When Osteoblasts were treated with medium conditioned by the mini screws/TADs for 24 h, cell viability was significantly decreased by all devices compared to the control. The number of dead cells present around the stainless-steel implants was higher compared to titanium-based implants, suggesting that titanium-based implants are more biocompatible when compared to stainless-steel implants.

DISCUSSION

The stability and biocompatibility of orthodontic mini-screws/TADs are vital to the clinical success of the product.^[11,12] Although the devices have a common composition of titanium,

few published reports have analyzed their cytotoxic potential. 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. In one study, there were no reported significant differences between human gingival fibroblast cell indexes of the control and study groups.^[13] In a study showing that stainless steel and titanium Grade IV and V mini-screws are biocompatible with immortalized human keratinocytes (HaCat), human osteogenic sarcoma cells (U2OS), and primary human gingival fibroblasts, the authors suggested that different cell lines may have varied response to be produced by mini-screws.^[14] Cytotoxic characteristics were observed after 24 h in both mini-screws. It is plausible that the MTT assay demonstrates a higher level of sensitivity in the cell culture system used for this study. A statistically significant decrease in cell viability after exposure to mini-screws/TADs incubated in saline at pH 4.0 has been reported.^[15] It is possible that initial solubilization of materials on the surface of new mini-screws/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 nontoxic levels. After this initial period, the data suggest that the TADs provide no detrimental effects to oral cavity tissues. In summary, the results shown here suggest that mini-screws/TADs examined in this *in vitro* study are titanium-based implants that are biocompatible with human osteoblasts.

CONCLUSION

The main aim of this study was to evaluate the biocompatibility of commercially available orthodontic mini-screws/TADs. Titanium-based implants showed less amount of cell death when compared to stainless steel implants when observed for 24 h. Long-term effect of these implants on the cell viability along with implants of different companies needs to be studied further.

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