

Comparison of biocompatibility of different materials of mini-implants: An in vitro study

Atika Ala Ansari¹ Chandrashekhar Varre² Gaffar Barood³ Samreen Jaweed⁴ Nikhila Sara⁵
Shivangi Gupta⁶

¹PG Student, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India,

²PG Student, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

³PG Student, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

⁴PG Student, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

⁵PG Student, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

⁶Reader, Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

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.

Materials and Methods: A cell culture system using commercially available human osteoblast cells was utilized. Mini screws/ temporary anchorage devices obtained from Revera Orthodontics were immersed in a culture medium for 24 hours. 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/temporary anchorage device conditioned culture medium. Biocompatibility and cytotoxicity were measured using a colourimetric 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.

Results: MTT analysis, used to quantify viable cells, demonstrated that 24-hour conditioned medium significantly affected cell viability.

Conclusion: When observed for 24 hours, 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; pathogenesis; management.

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 al² and Kanomi³ 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

Received: Apr. 6, 2024; Accepted: May. 20, 2024

*Correspondence Dr. Atika Ala Ansari.

Department of Orthodontics and Dentofacial Orthopaedics, Sri Balaji Dental College, Himayatnagar, Telangana, India.

Email: Not Disclosed

their small size allows more placement sites in the oral cavity and reduces bone-healing time⁴. 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 unfavourable 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.^{5,6,7,8} The material used to manufacture mini-implants must be nontoxic, biocompatible and mechanically resistant to support tension and corrosion.⁸ Therefore, commercially pure titanium (cp Ti) is the material of choice, as it is biocompatible and presents satisfactory mechanical properties.¹

Aim and Objective:

The objective of this study was to provide more evidence for the biocompatibility of 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 compounds¹⁰.

MATERIAL AND METHODS

Preparation of Mini screws

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

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

The mini-implants as received from the vendor were sterilized by ultraviolet light for 24 hours 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 48hours. The culture medium was collected for analysis after 24 hours before 5 ml of fresh medium was added. The devices were then incubated. Culture medium conditioned by the mini-implants for 48 hours and was sterile-filtered through a 0.22µm 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 hours at 37 °C in 95% air and 5% carbon dioxide. Cells were incubated in serum free medium only as the control as used as the measure of cell survival.

After the 24 hour 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 hours. 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%. Prior to initiating the MTT assay, 50 microliters of the culture medium was 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 ¹¹. 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 hours, 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 is vital to the clinical success of the product ^{12,13}. Although the devices have a common composition of titanium, few published reports have analysed their cytotoxic potential. To our knowledge based on a search of the literature, only two studies have been published to directly

analyse 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 14. 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 15. Cytotoxic characteristics were observed after 24 hours 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 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 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 hours. Long term effect of these implants on the cell viability along with implants of different companies needs to be studied further.

REFERENCES

1. Morais LS, Serra GG, Muller CA, Andrade LR, Palermo EF, Elias CN, . Titanium alloy mini-implants for orthodontic anchorage: immediate

loading and metal ion release. *Acta Biomater* 2007;3: 331-9.

2. Wehrbein H, Glatzmaier J, Yildirim M. Orthodontic anchorage capacity of short titanium screw implants in the maxilla. An experimental study in the dog. *Clin Oral Implants Res* 1997;8:131-41.

3. Kanomi R. Mini-implant for orthodontic anchorage. *J Clin Orthod* 1997;31:763-7.

4. Chen Y, Shin HI, Kyung HM. Biomechanical and histological comparison of self-drilling and self-tapping orthodontic microimplants in dogs. *Am J Orthod Dentofacial Orthop* 2008;133:44-50. 8. ISO 10993-5: 2009.

5. Block MS, Hoffman DR. A new device for absolute anchorage for orthodontics. *Am J Orthod Dentofacial Orthop*. 1995;107:251-258.

6. Sugawara J, Daimaruya T, Umemori M. Distal movement of mandibular molars in adult patients with the skeletal anchorage system. *Am J Orthod Dentofacial Orthop*. 2004;125:130-138.

7. Palagi LM, Sabrosa CE, Gava EC, Baccetti T, Miguel JA. Long-term follow-up of dental single implants under immediate orthodontic load. *Angle Orthod*. 2010;80:807-811.

8. Nishimura M, Sannohe M, Nagasaka H, Igarashi K, Sugawara J. Nonextraction treatment with temporary skeletal anchorage devices to correct a Class II Division 2 malocclusion with excessive gingival display. *Am J Orthod Dentofacial Orthop*. 2014;145:85-94

9. Sumantran VN. Cellular chemosensitivity assays: an overview. *Methods Mol Biol*. 2011;731:219-36.

10. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods*. 1983;65(1-2):55-63.

11. Papadopoulos MA, Tarawneh F. The use of miniscrew implants for temporary skeletal anchorage in orthodontics: a comprehensive review. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2007;103(5):e6-15.

12. Cho YC, Cha JY, Hwang CJ, Park YC, Jung HS, Yu HS. Biologic stability of plasma ion- implanted miniscrews. Korean J Orthod. 2013;43(3):120-6.
13. Malkoç S, Ozturk F, Corekci B, Bozkurt BS, Hakki SS. Real-time cell analysis of the cytotoxicity of orthodontic mini-implants on human gingival fibroblasts and mouse osteoblasts. Am J Orthod Dentofacial Orthop. 2012;141(4):419-26.
14. Galeotti A, Uomo R, Spagnuolo G, Paduano S, Cimino R, Valletta R, et al. Effect of pH on in vitro biocompatibility of orthodontic miniscrew implants. Prog Orthod. 2013;14:15.