ISSN: 2581-5989
PubMed - National Library of Medicine - ID: 101738774

*International Journal of Dental Science and Innovative Research (IJDSIR)*

*IJDSIR : Dental Publication Service*

*Available Online at: www.ijdsir.com*

*Volume – 6, Issue – 3, May - 2023, Page No. : 89 – 95*

# A histological and spectroscopic investigation into soft tissue found over submerged titanium implants.

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**Citation of this Article:** Siddharth Singhrour, Sonu Yadav, Arunava Saha, Mohd. Ayub Siddique, Vishwas Singh, "A histological and spectroscopic investigation into soft tissue found over submerged titanium implants", IJDSIR- May - 2023, Volume – 6, Issue - 3, P. No. 89 – 95.



**Type of Publication:** Original Research Article

**Conflicts of Interest:** Nil

## Abstract

The present investigation sought to determine the titanium content of the muco-periosteal flaps that covered immersed titanium implants. 38 biopsies that were performed between 2.4 and 18 months (mean: 5.7) following implant implantation made up the examined matter. Any impact of the implantation trauma itself was disregarded due to the obvious time lag between the implantation and biopsy. The manufacturers of the implants were HaTi. These implants' surface regions vary in size and composition. The surface changes did not appear to have any notable effects on the titanium impregnation of the studied biopsies. The following can be clarified through recognizing that the implant surface that made interface with the removed tissue was only the top diameter and not the entire implant surface. Titanium in the biopsies was analyzed in terms of its effect histologically and regarding the titanium quantity by spectrophotometry. Even the highest titanium contamination was without a negative effect on the mucoperiosteal cover flaps. A correlation between time delay between implantation and biopsy or of the titanium amount and tissue reactions was not demonstrable. In summary, the results again highlighted the biological acceptance of titanium.

**Keywords:** Spectrophotometry, Titanium; Soft tissue

## Introduction

Biomaterials utilised in clinical settings ought to be non-toxic, non-carcinogenic, non-allergenic, and non-radioactive. High mechanical durability is also required

in the instance of endosseous oral implants [1]. As a result, ceramic implants are now largely obsolete, and titanium implants of various forms predominate [2].

Because of titanium implants' great physiological acceptability and clinical practicability, cobalt, chromium, molybdenum, and tantalum are no more relevant implant constituents. Osborn [3] categorised the bio-response to endosseous implants into three categories: bio-tolerant, bio-inert, and bio-reactive. Distanceosteogenesis (bio-tolerant), contactosteogenesis (bio-inert), or a physicochemical interaction between the implant and surrounding bone (bioreactive) distinguishes these groupings. Such histologically detected implant incorporation categories are modified not only by surgical methods, but also by implant material [4,1]. We are now aware that the post-insertion healing time is more important than the material itself [5]. Even the most biocompatible materials will be separated from the bone by a fibrous membrane of varied thicknesses if this unloading healing does not occur. When titanium comes into contact with oxygen, it is instantly coated by a titaniumoxide layer (a-case), which begins as titanium monoxide and ends as rutile surface, titanium dioxide [6,2].

Rutile is characterised as a "stable crystalline structure with bioreactive properties akin to porcelain." [2]. Titanium degrades slowly due to its rutile surface, therefore titanium implants should not cause metallosis. Nonetheless, several studies have found titanium not just in the bone surrounding enosseous implants, but also in regional lymphatic nodules, as well as in the liver, kidney, and spleen [7-9].

Histological tissue examination is a standard approach used for assessing the biocompatibility of implant materials [10-17]. Furthermore, X-ray scanning spectrography and enzyme assays are discussed [15,18]. All of these procedures attempt to determine the impact of implanted material on live tissue surrounding these foreign things. There are occasionally relationships between the amount of titanium in tissues and the observed cellular reactions [13,16,18]. In general, the clinical significance of such observations is regarded as insignificant. All current research focus on the bone-implant contact zones, but no previous attempt has been made to provide information on the titanium in the muco-periosteal flap over buried implants.

**Methodology**

To accomplish unloading healing, 38 implants from various manufacturers were implanted into 34 patients and covered by a muco-periosteal flap during a time span of 2.4-18 months (mean: 5.9 months). The period required to osseointegrate implants in the mandible is defined at 3 months and 6 months in the maxilla [19]. The soft tissue above the implants was then excised with a trephine burr for bioptic examination. For fixing, the sample was immersed in a 10% puffered formalin solution. Following the standard dehydration technique, these biopsies were embedded in paraffin and microtome slices with a thickness of 3 mm were created. Three cuttings survived unstained from all areas for additional study. The remaining samples were dyed with HE, van Gieson, and Berlin Blue (Table 1). In six cases, we employed the Kardasewitsch reaction to exclude formalin pigments from preventing artefacts caused by the fixation procedure. The unstained cuttings are placed in a solution of NH4OH 1-5% in 70% ethylene alcohol to eliminate such artefacts. After 5 minutes to 4 hours, all formerly existent artefacts vanished without leaving any trace of the specific staining [20].

The leftover sample, which wasn't necessary for histology, which was used for induced coupled plasma

(ICP) emission spectroscopy to determine the titanium content of the material. As a result, all biopsy material was examined histologically as well as by ICP-emission spectroscopy. The ICP technique is regarded as a highly effective approach [18]. Because the procedure is based on a fluid material, the specimens are first ashed under pressure within an enclosed structure in an oxygen atmosphere. The ash is then dissolved and pumped into a hot core of argon plasma burning in a concentric silicon tube ("torch") powered by a high-frequency generator through an induction roll.

To spray in the ash solution, we used a concentric pneumatic technique with Pt/Ir-Capillaries (Jobin-Yvon) or ultrasound (Mod.UNSP-1, Plasma Therm Inc.) in combination with a peristatic suction pump working at 0.9 ml/min. Using this procedure, the investigated aerosol is exsiccated and the atomized particles expose not only the quality but also the quantity of the titanium via beam emission to the spectrometer. The ICP-spectrometer JY38P used was produced by Instruments S.A., Jobin-Yvon. The stimulating unit came from Plasma-Therm. The high-frequency generator had a maximum output of 1.5 kW and a frequency of 27.12 MHz. The spectrometer is thermostatizable, and it is coupled with a Czerny Turner model with a 1 m beam focus and a holographic net of 2400 lines/mm. The possible range for spectral analysis is 0.02 mm. A PDP-11/03 calculator system was used for the monochromatic evaluation.

**Results**

In all cases, iron-containing intracellular pigments were evident by staining with the Berlin Blue reactions (Figs. 1 and 2). There were also black particles of varying sizes (Fig. 3). The lack of a repulse reaction in the tissue around these foreign bodies was obvious. Signs of inflammation characterized by macrophages, lymphocytes and plasma cells around these irritants were mostly mild [21,4,22] (Figs. 4–6). In the epithelium, an orthokeratotic reaction was the norm (Fig. 7). There were also keratohyaline granules and at the surface there were keratinous cells without nuclei. Some cases were with isolated epitheliae islets. A possible correlation was sought between implant types, delay after insertion and the level of inflammation (Figs. 8–10). The inflammation was graded based on the cellular elements: Grade I=none, Grade II=low grade infiltration, Grade III=medium grade infiltration and Grade IV=high amount of cell infiltration.

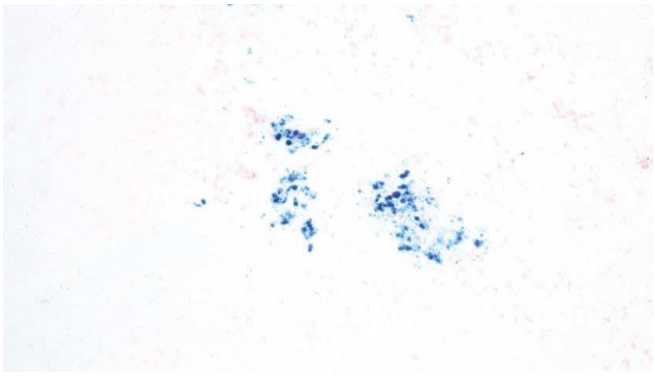

Fig. 1: Iron incorporation demonstrated by Berlin Blue reaction

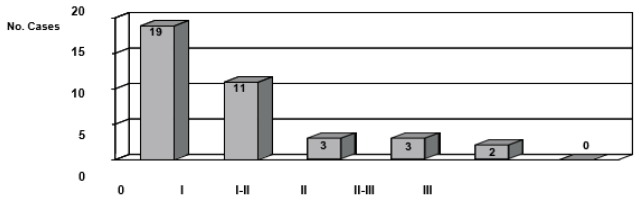


Fig. 2: Graph). Distribution of the observed case and their grade of Fe+ containing.

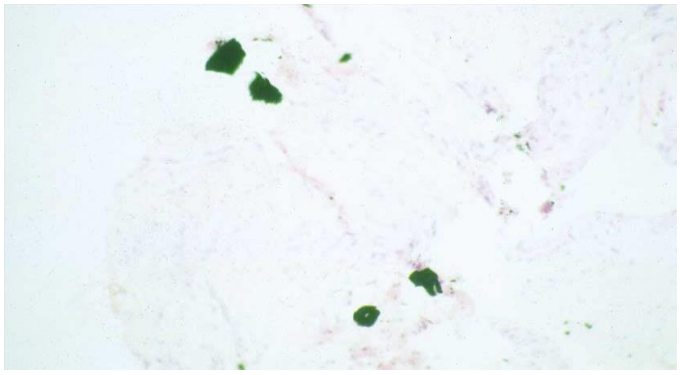

Fig. 3: Foreign particles grade II–III in the covering soft tissue excision (magn. 4 ×).

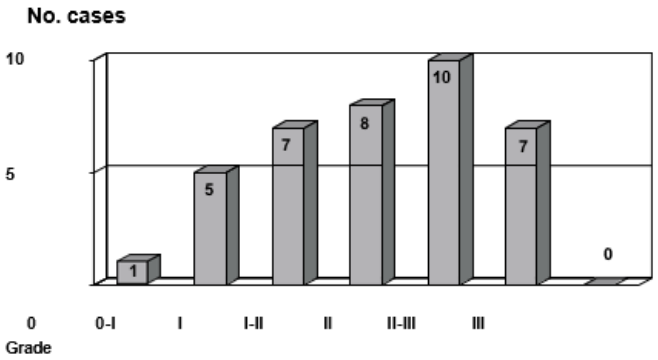


Fig. 4: (Graph). Distribution of the found chronical inflammations of the periimplant soft tissues according to grades.

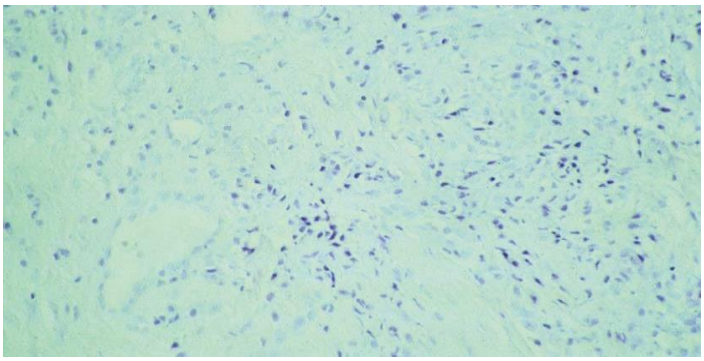

Fig. 5: Inflammatory reaction grade I–III, zones of granulation tissue rich in capillaries and fibroblasts (magn. 40 ×).

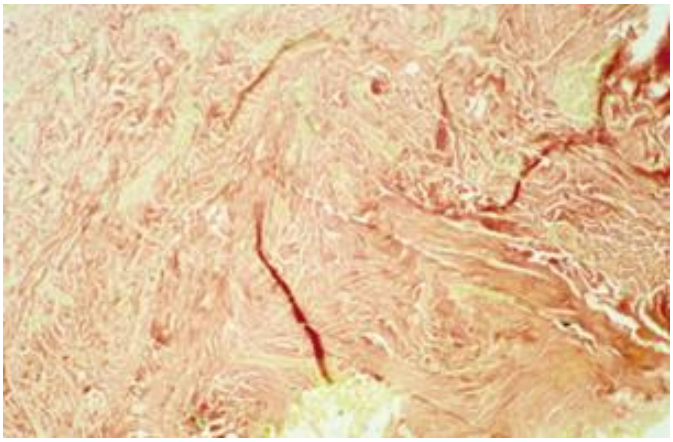

Fig. 6: Fibrosis grade III as a result of chronic inflammatory reaction (magn. 40 ×). grades of imflammation (1-3)

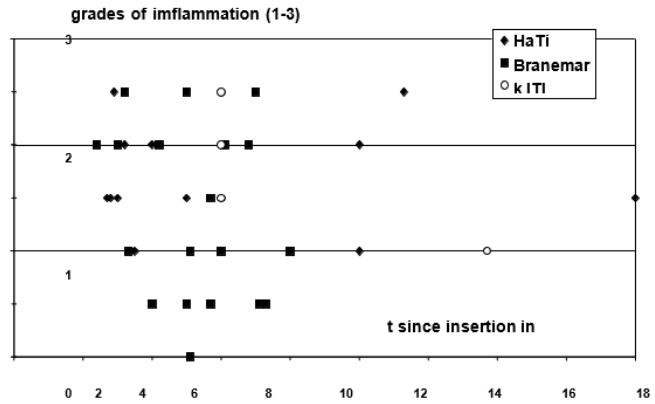


Fig. 7: (Graph). Correlation between implant types, time since insertion and level of inflammation found in the soft tissues. titanium concentration in mg / kg biopsy

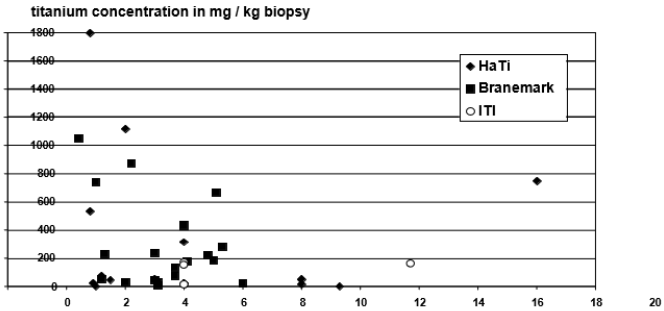


Fig. 8: (Graph). Correlation between implant types, time since insertion and titanium concentration found in the surrounding soft tissues.

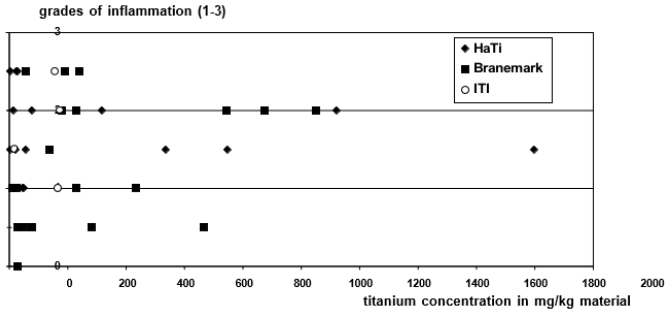


Fig. 9: (Graph). Correlation between implant types, time since insertion and titanium concentration found in the surrounding soft tissues.

**Discussion**

Titanium is widely known for its good biocompatibility [19,11]. While titanium alloys such as Ti6A14V are utilised in the United States [14], Europeans prefer pure titanium, which was employed in our study [2]. Corrosion products from metallic components are frequently an irritant upon implantation [23]. Because of the ceramic-like layer (rutile) on pure titanium surfaces, corrosion-related issues with such pure titanium implants have yet to be described. No main wound-healing effects are predicted because our samples were obtained at least 2.4 months following implantation. Rather, we only come across conventional material-related reactions. In general, there was no correlation between inflammatory levels, titanium concentration, and insertion time. Study demonstrates pure titanium's high bio-acceptance. Also, when he measured the amount of macrophages, leucocytes, neutrophilic granulocytes, and granuloma-like chronic inflammatory reactions, Perren [24-26] could not find such an association. Notwithstanding the considerable surface differences, the degrees of inflammation detected and connected to the different implant products are roughly identical to those of lTI (Straumann, Switzerland) 1.7, HaTi (Matthys, Switzerland) 1.8, and Branemark 1.3. The soft tissue flap above the 1.7, HaTi (Matthys, Switzerland) 1.8 and Branemark (Nobelbiocare, Sweden) 1.3 despite the impressive surface differences. The soft tissue flap above the implants may not have covered the implant diameter completely over the observation time.

Small leaks are frequently undetectable clinically, but they can produce a mild persistent illness. The epithelial reactivity of the flaps was generally orthokeratotic, but there were also a few medium in size epithelial hyperplasias that could be connected to such leaks. The content of titanium in the biopsies varied greatly (Figs. 9 and 10). The average amount of discovery in HaTi (15 instances) was 322.19 mg, 127.28 mg in ITI material (4 cases), and 290.11 mg in Branemark (19 cases) (Fig. 9).

It is challenging to explain this series of average titanium concentration because the ITI surface is increased about 10-fold by plasma flame spraying. However, we must remember that only the surface diameter was in touch with the biopsied areas. Variations in titanium composition could be caused by mechanical irritations inflicted on implants during insertion by steel devices [27], an explanation proposed before by Fischer-Brandies et al. [8] and Schliephake et al. [9]. We must assume that the implant-covering flap was never absolutely immobile and the existing small movement may have led to an eraser-like effect which impregnated the tissue continuously with titanium. Since the titanium transfer to the muco-periosteal flap during insertion is only a minor possibility due to the standardized surgical technique, the ‘‘eraser’’ effect may be the only remaining explanation.

**References**


1. Williams DF. Biocompatibility of clinical implant materials. New York: CRC Press, 1971
2. Steinemann S. Werkstoff Titan. In: Schroeder A, Sutter F, Krekeler G, editors. Orale Implantologie. Stuttgart: Thieme, 1988
3. Osborn JF, Kovacs E, Kallenberger A. Hydroxylapatitkeramik- Entwicklung eines neuen Biowerkstoffes und erste tierexperimen- telle Ergebnisse. Dtsch Zahna.rztl Z 1980;35:54–6.
4. Anderson JM, Miller KM. Biomaterial, biocompatibility and the macrophage. Biomaterials 1984;5:5–10.
5. Donath K, Kirsch, A. Welche Bedeutung hat die prima. re Stabilisation von Implantaten fu. r die ossa.re Integration wa. hrend der Einheilphase? Dtsch Z Zahna.rztl Implantol II 1986:11–17.
6. Kasemo B. Biocompatibility of titanium implants: surface science aspects. J Prosthet Dent 1983;49:832–7.
7. Ferguson AB, Akahoshi Y, Lang P, Hodge ES. Trace metal ion concentration in liver, kidney, spleen and lung of normal rabbits. J Bone Jt Surg 1962;44:317–22.
8. Fischer–Brandies E, Zeintl W, Schramel P, Brenner K-U. Zur Frage der Gewebebelastung mit Titan nach Schraubenosteosynthese. Dtsch Z Mund-Kiefer-Gesichts-Chir 1993;17:93–4.
9. Schliephake H, Reiss J, Urban R, Neukam FW, Guenay H. Freisetzung von Titan aus Schraubenimplantaten. Dtsch Z Zahn.arztl Implantol 1991;7:6–10.
10. Bagnall RD. An approach to the soft tissue/synthetic material interface. J Biomed Mater Res 1977;11:939–46.
11. Donath K. Klinische und histopathologische Befunde im Implantatlagergewebe bei Titan Implantaten. ZWR 1987;96:14–6.
12. Ferguson AB, Laing P, Hodge ES. The ionization of metal implants in living tissues. J Bone Jt Surg 1960;42:77–90.
13. Laing PG, Ferguson AB, Hodge ES. Tissue reaction in rabbit muscle exposed to metallic implants. J Biomed Mater Res 1967;1:135-49
14. Parr GR, Gardner LK, Toth RW. Titanium: the mystery metal of implant dentistry dental material aspects. J Prosthet Dent 1985;54:410–4.
15. Rae T. A study on the effects of particulate metals of orthopaedic interest on murine macrophages in vitro. J Bone Jt Surg 1975; 57:444–50.
16. Rae T. The biological response to titanium and titanium– aluminium-vanadium alloy particles. Biomaterials 1986;7:30–6.
17. Riede UN, Ruedi T, Limacher F. Quantitative und morphologische Erfassung der Gewebereaktion auf Metallimplantate (Osteosynthesematerial). Arch Orthop Unfall–Chir 1974;79: 205–15.
18. Schramel P, Klose B-J, Hasse S. Die Leistungsf.ahigkeit der ICPEmissionsspektroskopie zur Bestimmung von Spurenelementen in biologisch-medizinischen und in Umweltproben. Fresenius Z Anal Chem 1982;310:209–16.
19. Branemark, et al. Osseointegrated implants in the treatment of the edentulous jaw: experience from a year period. Stockholm: Almquist u. Wiksell, 1977.
20. Gotfredsen K, Budtz–Joergensen E, Nimb Jensen L. A method for preparing and staining histological sections containing titanium implants for light microscopy. Stain Technol 1989;64:121–7.
21. Adams DO. The granulomatous inflammatory response. Am J Pathol 1976;84:164–91.

22. Coleman D, King RN, Andrade JD. The foreign body reaction: a chronic inflammatory response. J Biomed Mater Res 1974;8:199–211.
23. Barbosa MA. Corrosion of Metallic Implants. In: Handbook of Biomaterial Properties. London, Weinheim, New York, Tokyo, Melbourne, Madras: Chapman & Hall, 1998
24. Perren SM. A dynamic compression plate. Acta Orthop Scand 1969;125:7–16.
25. Donath K, Laass M, Guenzl H-J. The histopathology of different foreign-body reactions in oral soft tissue and bone tissue. Virchows Arch A 1992;420:131–7.
26. Strassl H. Experimentelle Studie .uber das Verhalten von titanbeschichteten Werkstoffen hinsichtlich der Gewebskompatibilita. t im Vergleich zu anderen Metallimplantaten. O. st Z Stomat 1978;75:82–98.
27. Weber H, Sauer K-H, Geis-Gerstorfer J, Kratzenstein B. Zur Metallaufnahme durch implantologische und prothetische Ma_nahmen. Z Zahn.arztl Implantol 1986;2:6–67.