Improvement of biohistological response of facial implant materials by tantalum surface treatment

탄탈륨 표면 처리에 의한 안면 임플란트 재료의 조직생물학적 기능 향상

2019년 2월

서울대학교 대학원

치의과학과 구강악안면외과 전공

모하마드 바크리 (Bakri Mohammed Mousa H)

Improvement of biohistological response of facial implant materials by tantalum surface treatment

탄탈룸 표면 처리에 의한 안면 임플란트 재료의 조직생물학적 기능 향상

지도교수 이종호

이 논문을 치의과학석사 학위논문으로 제출함

2018 년 **10** 월

서울대학교 대학원

치의과학과 구강악안면외과 전공

모하마드 바크리 (Bakri Mohammed Mousa H)

모하마드 바크리 (Bakri Mohammed Mousa H) 의

석사 학위논문을 인준함

2018년 12

위 원 장   김현이   (인)
부위원장   이종호   (인)
위   원   김봉주   (인)

<div align="center">Abstract</div>

Improvement of biohistological response of facial implant materials by tantalum surface treatment

<div align="right">

Bakri Mohammed Mousa H,

Program in Oral and Maxillofacial Surgery, Department of Dental Science,

Graduate School

Seoul National University

</div>

Purpose

A compact passive oxide layer can grow on tantalum (Ta). It has been reported that this oxide layer can facilitate bone ingrowth under in vivo conditions though the development of bone-like apatite, which promotes hard and soft tissue adhesion (Valencia-Lazcano et al., 2018). Thus, tantalum surface treatment on facial implant materials may improve tissue response, which results in less fibrotic capsule encapsulation and makes the implant more stable on the bone surface. The purpose of this study was to verify whether surface treatment of facial implant materials using tantalum can improve the histobiological response and to determine the possibility of potential clinical application.

Materials and Methods

Two different commonly used implant materials [i.e., silicone and expanded polytetrafluoroethylene (ePTFE)] were treated via Ta ion implantation using a Ta sputtering gun. Ta-treated samples were compared with untreated samples using in vitro and in vivo evaluation. Osteoblast (MG-63) and fibroblast (NIH3T3) cell viability on the Ta-treated implant material was assessed, and tissue response was observed by placing the implants over the rat calvarium (n = 48) for two different time intervals. Foreign body and inflammatory reactions were observed, and soft tissue thickness between the calvarium and the implant as well as the bone response were measured.

Results

The treatment of facial implant materials using tantalum showed a tendency toward increased fibroblast and osteoblast viability, although it was not statistically significant. During the in vivo

study, both Ta-treated and untreated implants showed similar foreign body reactions. However, Ta-treated implant materials (silicone and ePTFE) showed a tendency toward better histological features: lower soft tissue thickness between the implant and the underlying calvarium as well as increased new bone activity.

Conclusion

Tantalum surface treatment using ion implantation on silicone and ePTFE facial implant materials showed the possibility of reducing soft tissue intervention between the calvarium and the implant to make the implant more stable on the bone surface. Although no statistically significant improvement was observed, tantalum treatment revealed a tendency toward the improved biohistological response of silicone and ePTFE facial implants. Conclusively, tantalum treatment is beneficial and has a possibility for potential clinical application.

Improvement of biohistological response of facial implant materials by tantalum surface treatment

Bakri Mohammed Mousa H, DDS

Program in Oral and Maxillofacial Surgery, Department of Dental Science,

Graduate School, Seoul National University

(Directed by Professor Jong-Ho Lee, DDS, MSD, PhD)

CONTENTS

I. Introduction

Facial augmentation procedures are some of the most commonly performed cosmetic procedures (Osman and Swain, 2015). Silicone rubber and silicone rubber-based materials are widely used in facial cosmetics. This has been the case for many years. However, there is an increasing evidence suggesting that the intrinsically hydrophobic nature of silicone rubber surface leads to poor cell adhesion and tissue compatibility between the implant and surrounding tissues, which results in capsule formation and gradual thickening and contracture of these tissues (Fischer et al., 2015, Wick et al., 2010). In addition, these capsular voids encourage bacterial infection and invasion as well as inflammation during long-term use.

If necessary, e-PTFE is relatively easy to remove. However, an unsatisfactory appearance may result from implant folding on itself due to mobility of the underlying tissues. Therefore, major disadvantages of using facial implants continue to be susceptibility to infection and possible displacement. Thus, there is a need for further research into ways of improving the outcome of facial implant use. Surface modification of implant materials is a commonly used method to improve biocompatibility of implant materials and to overcome the abovementioned disadvantages. Various materials have been used as implant coatings.

Tantalum is receiving increasing interest as biomaterial due to its excellent biocompatibility (e.g., outstanding bone-like apatite forming capability, absence of cytotoxic ion release or dissolution in local, systemic and remote organs, as well as good osseointegration), superior strength, and anti-corrosion properties (Huo et al., 2017). Tantalum is a corrosion-resistant transition metal element with atomic number 73. It is a promising metallic material, at least in terms of bioperformance. It has been used in medical field since 1940s when Burke used pure tantalum in several cases such as in skin, subcutaneous and tendon sutures as well as in several plates (Romo et al., 2002). Tantalum has an ability to form a compact, passive, extremely thin and transparent but strong and tenacious oxide layer that strongly adheres to tantalum. This oxide layer has a capacity to facilitate bone in-growth under in vivo conditions via the development of bone-like apatite that promotes hard and soft tissue adhesion (Levine et al., 2006). Furthermore, Ta is a hard, ductile and highly chemical resistant material with good apposition to human bone. In addition, mechanical properties of tantalum are impressive. The metal is comparable to steel in its strength, toughness and workability (Li et al., 2013) (Stenlund et al., 2015). The aim of this study was 1) to verify whether the use of

tantalum as a surface treatment material for facial implant materials can improve histobiological features and 2) to determine the possibility for potential clinical application.

II. Materials and Methods

Tantalum ion implantation:

A 0.85-mm thick ePTFE membrane (Meari Co., Ltd., Gyeonggi-do, Republic of Korea) and a 1-mm thick silicone rubber consisting of clinical grade silicone (Bistool Co. Ltd., Seoul, Republic of Korea) with dimensions of 10×10 mm were prepared for the experimental evaluation. All samples were ultrasonically cleaned in alcohol and deionized water for 5 min before tantalum (Ta) coating processing. A Ta target (diameter 75 mm, thickness 5 mm, purity 99.99 %, Kojundo Korea Co., Ltd., Gyeonggi-do, Republic of Korea) was placed in a DC magnetron sputter gun housing (Ultech Co. Ltd., Daegu, Korea). The vacuum chamber was pumped to 5×10^{-4} Pa using rotary and diffusion pumps. To generate a sufficient amount of Ta ions and neutral atoms, 25 W of target power was applied to the Ta sputtering gun, and a working pressure and temperature were maintained, respectively, at 0.6 Pa and 25°C during the process. The samples were placed on a stainless-steel plate parallel to the Ta target surface at a 100-mm distance from it. Ta ions and neutral atoms were implanted into the sample surfaces for 3 min using a high negative bias of 2000 V. For comparative purposes, only untreated ePTFE and untreated silicone rubber sheets were used for the control group. Thus, all of the experiments in this study were divided into the following four groups: Ta-treated silicone implant (G1), untreated silicone implant (G2), Ta-treated ePTFE implant (G3) and untreated ePTFE implant (G4). Tantalum ion implantation into implant materials was performed at the Department of Material Science and Engineering, Seoul National University, Seoul, Korea.

TEM and SEM observation:

To observe the Ta-implanted regions on implant surfaces, high-resolution transmission electron microscope images were collected using a transmission electron microscope (TEM) (JEM-2100F, JEOL, Japan) operated at 200 kV. The cross-sectional image of the Ta-coated implant surface was prepared and obtained using focused ion beam milling and field-emission scanning electron microscopy (FIB/FE-SEM) (AURIGA, Carl Zeiss, Germany). Prior to the milling process, protective layers containing platinum and carbon were coated onto the implant surfaces.

Cells viability:

   To evaluate the viability of Osteoblast (MG-63) and Fibroblast (NIH3T3), a EZ-Cytox assays Daeil Lab Service Co. Std, Seoul, Korea) were performed according to the manufacturer's protocol. MG-63 and NIH3T3 were plated at a density of 3×10^4 cells/mL on implant materials, and cultured in Dulbecco's modified eagle medium (DMEM, ATCC 30-2003) (Life Technologies Co., Grand Island, NY, USA) and Eagle's Minimum Essential Medium (EMEM, Gibco 11995) (Life Technologies Co.) with 10% fetal bovine serum (FBS) containing 1% penicillin/streptomycin at 37 °C under 5% CO_2 in a humidified atmosphere, respectively. Implants were conditioned with in a medium for 5 hours before insertion to 24 well cell culture plates. After cultured for 24, 48 and 72, h, the culture medium was discarded, and the samples were washed thrice with PBS then incubated at 37 °C for another 4 h in fresh culture medium containing 10 $\mu\ell$ of EZ-Cytox solution. To investigate the effects of the tantalum-treated or untreated silicone or ePTFE surface on the viability of MG-63 and NIH3T3, the absorbance values of each cell culture were measured by using a spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) at 490 nm. Each test was repeated four times (n = 6).

Animal study:

   For biohistological evaluation, an animal experiment was conducted. All experimental surgical procedures were performed at the Specific Pathogen-free unit of the Animal Facility Laboratory at the School of Dentistry, Seoul National University, Seoul, Republic of Korea. Ethical approval was obtained from the Institutional Animal Care and Use Committee (Approval number: SNU-180328-1). The animals, six-week-old male healthy Sprague-Dawley rats (SD), were kept in a room with a 12 h light/dark cycle and temperature that varied between 23 and 25 °C. Furthermore, the animals were housed in soft, sterile bedding that was free from antibacterial products. The animals had open access to food and sterile non-acidic water.

 The rats were randomly assigned to one of the following groups:

G1 (n = 6): Tantalum-treated silicone implant,

G2 (n =6): Untreated silicone implant,

G3 (n = 6): Tantalum-treated ePTFE implant,

G4 (n = 6): Untreated ePTFE implant.

The experiment was conducted in two time intervals: 4 and 8 weeks for the silicone implant material and 2 and 4 weeks for the ePTFE implant material. The animals were anesthetized using a Ketamine/Xylazine mixture (75-100 mg/kg of Ketamine + 5-10 mg/kg of Xylazine), which was administered intraperitoneally (IP) using a maximum dose of 10 mL/kg. The incision site, which is located behind the lambdoid suture, was shaved and painted using iodine swabs. An approximately 2-cm-long incision was made behind the lambdoid suture through the skin, subcutaneous tissue, deep fascia and periosteum up to the calvarial bone. Soft tissue over the skull bone was reflected, and the implants were inserted for all animals in all 4 groups (Fig. 1). Skin apposition was achieved using subcutaneous sutures made with 4-0 vicryl (Ethicon, Livingston, UK).

Histological evaluation:

At the completion of the study, the rats were sacrificed using an overdose of an intraperitoneal Ketamine/Xylazine mixture. Histologic samples, including implants and surrounding tissues, were obtained carefully to prevent implant movement. The samples were fixed in buffered formalin for 24 h, dehydrated and embedded in paraffin wax. Tissue sections were mounted on glass slides and stained with Hematoxylin and Eosin (H&E) for histopathological evaluation. Images were captured using a specialized system, SPOT RTTM-KE color mosaic, and digitized via the SPOT software version 4.6 (Diagnostic Instruments, Inc., Sterling Heights, MI, USA). For simplicity, images were studied at 400× magnification. Soft tissue thickness between the implants and the bone, the extent of new bone formation and the severity of inflammatory reaction were measured and analyzed. The sections were processed, placed on slides and stained with hematoxylin and eosin stain. Histology evaluation was performed on each section to evaluate inflammation, foreign body reaction, amount of soft tissue filling the gap between the implants and the calvarial bone, and newly formed bone along the superficial layer of the calvarium toward the implants. Inflammation and foreign body reaction were evaluated blinded by a board-certified pathologist. According to the method of Pinese et al. (2018), soft tissue measurements were taken at 13 random regions along the implant bone gap and then averaged to compare between Ta-treated and untreated implants (Pinese et al., 2018). To evaluate the newly formed bone, histological evaluation of the histological slides was conducted. The newly formed bone was evaluated and scored according to its quantity. The following scores were assigned, as appropriate: no bone (score = 0), little stumps of bone (score = 1), moderate bone

with gaps (score = 2) and complete bone along the calvarium surface (score = 3) (Schallenberger et al., 2014).

Statistical analysis:

All data were expressed as the mean ± standard deviation (SD). Data analysis was conducted using the SPSS Statistics software ver. 25 (IBM, Armonk, NY, USA). Nonparametric data comparisons were performed using the Wilcoxon-Mann-Whitney U test. Statistical significance was set as p < 0.05.

III. Results

Tantalum ion implantation:

After surface modification via Ta ion implantation, the Ta element was observed on the surface of implant materials. In TEM cross-sectional images (Fig. 2), a Ta-implanted region with a 20~30-nm thickness was clearly detected between the surfaces of implants and the protective carbon coating layer.

 Cell viability:

The mean absorbance value at a wavelength of 450 nm for Ta-treated silicone implant materials was 0.060 ± 0.013, and for untreated silicone implants, the value was 0.054 ± 0.040. The OD was higher for the Ta-treated silicone implant material, however the result was not statistically significant (p-value = 0.4). The ODs for Ta-treated silicone and for untreated silicone were, respectively, 0.089 ± 0.034 and 0.062 ± 0.023, p-value = 0.4. The result was better than that for untreated silicone, but not statistically significant (Table 1).

Histological evaluation:

All animals recovered uneventfully after the implantation. There were no cases of death, swelling, or pus discharge at the implant sites in any of the animals during the study period. According to a report by a pathologist from the Seoul National University School of Dentistry, macrophages and gain cells were observed in a short-term experiment. However, in a long-term experiment, none of these cells were observed. Soft tissue filling the gap between the implant and the calvarium bone was evaluated in all specimens, and the mean $\pm$ standard deviation (SD) was recorded. Comparisons were performed using the Wilcoxon-Mann-Whitney U test. For short-term Ta-treated silicone implants, the mean soft tissue thickness was 73 ± 34 μm, while the mean thickness for the untreated silicone implant was 92 ± 43 μm. There was less soft tissue filling the gap for the Ta-treated implant material, however the result was not statistically significant (p-value = 0.7). In case of long-term experiment of silicone implant materials, the Ta-treated implant showed better result compared with untreated implants (Ta-treated: 82 ± 27 μm and untreated: 115 ± 0.24 μm). However, this result was not statistically significant (p-value = 0.3). In case of the newly formed bone in the short-term silicone implant material, we did not observe any newly formed bone either in Ta-treated silicone implants or in untreated silicone implant materials. However, in long-term experiment, a similar amount of newly formed bone was observed in

Ta-treated and untreated silicone implants (0.20 ± 0.45 μm, p-value = 1.00). Ta coatings did not show a significant improvement compared with the untreated surface (Table 2) (Fig. 3).

In case of ePTFE implant materials, in the Ta-treated group, the mean soft tissue thickness was 95 ± 71 μm, while the mean thickness for the untreated ePTFE implant was 111 ± 70 μm in short-term experiment. There was less soft tissue filling the gap in case of the Ta-treated implant material, however the result was not statistically significant (p-value = 0.6). In case of long-term experiment of ePTFE implant materials, the Ta-treated implant did not show any statistically significant differences. For the Ta-treated material, the mean soft tissue thickness was 38 ± 17 μm, and for untreated implants, the mean soft tissue thickness was 70 ± 63 μm (p-value = 0.7). In case of the newly formed bone evaluation, in short-term experiment, the newly formed bone score was 0.60 ± 0.55 for Ta-treated implant materials, and 0.50 ± 0.58 for untreated implant materials. The p-value was 0.80, which mean that our result is not statistically significant. In terms of long-term experiment, the Ta- treated implant material had a higher amount of newly formed bone (1.40 ± 0.89) compared with the untreated implant material (1.33 ± 1.15). However, the result is not statically significant (p-value = 0.9).

IV. Discussion

Surface treatment of facial implant materials using tantalum (Ta) is considered as a promising surface modification technique. Tantalum treatment is of significant interest because it is possible to use tantalum as a surface treatment material via cold spray ion implantation technique. Cold spray has several advantages over other surface treatment techniques. Specifically, it results in little or no oxidation during material build-up, and it produces a dense coating. The process can provide a relatively high deposition efficiency, and the process is conducted in a cold environment, which minimizes any deleterious effects on the treated material. These unique characteristics explain the current popularity of tantalum surface treatment (Levine et al., 2007).

Many studies have used surface modification to enhance biocompatibility of medical devices (drug-eluting stents, artificial organs, biosensors, catheters, scaffolds for tissue engineering, heart valves, facial augmentation materials, etc.). In orthopedics, tantalum surface treatment is considered as a promising surface modification technique. It shows good effect on cell viability and differentiation. In clinical applications, tantalum has been used as a coating material and showed ability to promote bone ingrowth (Wang et al., 2016). Several studies have proven that tantalum surface treatment has an ability to improve surface mechanical properties and osteogenic activity of orthopedic devices. However, there are no studies on Ta-treated facial materials (Huo et al., 2017).

In this study, two commonly used facial implant materials (silicone and ePTFE) were treated using Ta ion implantation. Silicone is frequently used to augment facial defects. Although it is pliable and easily shaped, it still has some disadvantages that lead to facial implant failure such as implant displacement and possibility of infection. Gore-Tex is a trade name of expanded polytetrafluoroethylene (ePTFE). It is safe to use long-term with very rare instances of rejection. Furthermore, ePTFE may be used as a guide membrane. However, a disadvantage of this material is its tendency to fold on itself, which leads to infection (Patel and Brandstetter, 2016). Despite the improvements in facial augmentation techniques, failures and complications still occur (Brügger et al., 2015).

In this experiment, using in vitro study and optical density measurements, the result of fibroblast cell viability for the Ta-treated silicone (0.060 ± 0.013) implant compared with the untreated implant (0.054 ± 0.008) was found to be statistically insignificant (p-value: 0.4). In addition, statistically insignificant result was observed for ePTFE implant materials.

Regardless of whether the result is or is not statistically significant, it is a good result because it proves that tantalum is not harmful to cells.

In this study, osteoblast viability assays were conducted as well. Neither the Ta-treated silicone implant material nor the Ta-treated ePTFE implant material was significantly better than the corresponding control groups. However, the mean osteoblast OD value of Ta-treated implant materials (silicone: 0.06, ePTFE: 0.09) was better than the mean OD values of the untreated implant material (silicone: 0.05, ePTFE: 0.06). However, in general, the result of the Ta-treated material (silicone and ePTFE) was better than that of the untreated implant material. According to a previous result, there was no negative effect on cell viability when using tantalum as a coating material. Researchers have determined that surface topography of an implanted material is important for morphogenesis. In addition, surface topography affects the biological behavior of cultured cells such as cell viability and differentiation (Filová et al., 2009).

In the absence of a foreign body, tissue trauma triggers a series of events that comprise wound healing, i.e., inflammation, viability and remodeling. Presence of a foreign body interferes with natural biological response and disrupts the healing process. As a result, we observe a foreign body reaction. The most common histological signs of a foreign body reaction are: increase of macrophages and gain cell formation, increase of fibroblast activity, and fibrous encapsulation of the foreign body (Kastellorizios et al., 2015). In our study, during daily post-operative period, zero rats showed signs or symptoms of an allergic reaction (e.g., weight loss, delayed wound healing, implant loss, dehiscence or any other signs).

During histological examination, macrophages and giant cells were observed in short-term experiments. However, these histological finding were absent in long-term experiment. It is a normal body reaction after a wound or a cut (Joe et al., 2004). During healing and during the inflammatory phase, migration of blood cells (e.g., phagocytic neutrophils and macrophages) to the wound site is a healthy physiological behavior (Koh and DiPietro, 2011). Moreover, macrophages were not associated with any other clinical finding. This means that it was just a normal response to surgical intervention.

Capsule contracture is considered to be an inevitable complication of using silicone implants (Kjøller et al., 2001). In this study, soft tissue filling the gap between the implant and the underlying bone was measured. Lower thickness means less soft tissue deposition and more completability. In addition, the amount of fibrous tissue in a small layer of soft tissue will be lower than its amount in a thick soft tissue layer. With no statistical significance, soft tissue thickness in Ta-treated implant materials was lower than that in the control groups

(untreated implant materials). Even though the result is not biostatistically strong, we can assume that Ta treatment prevents fibrous capsule formation. It is possible that this advantage is due to implant's hydrophilic nature, which was acquired after the Ta treatment.

In this study, soft tissue thickness in the 4-week ePTFE implant material study was lower compared with 2-week implants. This means that not all soft tissue is due to fibrotic band. Thus, long-term experimental study is recommended to verify whether we can achieve definitive results regarding the thickness change of soft tissue after the tantalum treatment. In case of the silicone implant material, even though the result of soft tissue thickness of the Ta-treated group (73 ± 34) was better than that of the untreated group (92 ± 43) in a 4-week observation, the 8-week observation was not better than that of a 4-week study.

V. Conclusion

Tantalum surface treatment using ion implantation on silicone and ePTFE facial implant materials showed the possibility of reducing soft tissue intervention between the calvarium and the implant, which results in the implant being more stable on the bone surface. Although statistically significant improvement was observed only for fibroblast viability on the Ta-treated implant, tantalum treatment revealed a tendency toward improving biohistological response of silicone and ePTFE facial implants. Conclusively, tantalum treatment is beneficial and has a possibility for potential clinical application.

Conflict of interest:

The authors declare that they have no conflict of interest.

References

Brügger, O. E., Bornstein, M. M., Kuchler, U., Janner, S. F., Chappuis, V. and Buser, D. (2015) 'Implant therapy in a surgical specialty clinic: an analysis of patients, indications, surgical procedures, risk factors, and early failures', Int J Oral Maxillofac Implants, 30(1), pp. 151-60.

Filová, E., Bullett, N. A., Bacáková, L., Grausová, L., Haycock, J. W., Hlucilová, J., Klíma, J. and Shard, A. (2009) 'Regionally-selective cell colonization of micropatterned surfaces prepared by plasma polymerization of acrylic acid and 1,7-octadiene', Physiol Res, 58(5), pp. 669-84.

Fischer, S., Hirche, C., Reichenberger, M. A., Kiefer, J., Diehm, Y., Mukundan, S., Alhefzi, M., Bueno, E. M., Kneser, U. and Pomahac, B. (2015) 'Silicone Implants with Smooth Surfaces Induce Thinner but Denser Fibrotic Capsules Compared to Those with Textured Surfaces in a Rodent Model', PLoS One, 10(7), pp. e0132131.

Huo, W. T., Zhao, L. Z., Yu, S., Yu, Z. T., Zhang, P. X. and Zhang, Y. S. (2017) 'Significantly enhanced osteoblast response to nano-grained pure tantalum', Sci Rep, 7, pp. 40868.

Joe, B., Vijaykumar, M. and Lokesh, B. R. (2004) 'Biological properties of curcumin-cellular and molecular mechanisms of action', Crit Rev Food Sci Nutr, 44(2), pp. 97-111.

Kastellorizios, M., Tipnis, N. and Burgess, D. J. (2015) 'Foreign Body Reaction to Subcutaneous Implants', Adv Exp Med Biol, 865, pp. 93-108.

Kjøller, K., Hölmich, L. R., Jacobsen, P. H., Friis, S., Fryzek, J., McLaughlin, J. K., Lipworth, L., Henriksen, T. F., Jørgensen, S., Bittmann, S. and Olsen, J. H. (2001) 'Capsular contracture after cosmetic breast implant surgery in Denmark', Ann Plast Surg, 47(4), pp. 359-66.

Koh, T. J. and DiPietro, L. A. (2011) 'Inflammation and wound healing: the role of the macrophage', Expert Rev Mol Med, 13, pp. e23.

Levine, B., Della Valle, C. J. and Jacobs, J. J. (2006) 'Applications of porous tantalum in total hip arthroplasty', J Am Acad Orthop Surg, 14(12), pp. 646-55.

Levine, B., Sporer, S., Della Valle, C. J., Jacobs, J. J. and Paprosky, W. (2007) 'Porous tantalum in reconstructive surgery of the knee: a review', J Knee Surg, 20(3), pp. 185-94.

Li, X., Wang, L., Yu, X., Feng, Y., Wang, C., Yang, K. and Su, D. (2013) 'Tantalum coating on porous Ti6Al4V scaffold using chemical vapor deposition and preliminary biological evaluation', Mater Sci Eng C Mater Biol Appl, 33(5), pp. 2987-94.

Osman, R. B. and Swain, M. V. (2015) 'A Critical Review of Dental Implant Materials with an Emphasis on Titanium', Materials (Basel), 8(3), pp. 932-958.

Patel, K. and Brandstetter, K. (2016) 'Solid Implants in Facial Plastic Surgery: Potential Complications and How to Prevent Them', Facial Plast Surg, 32(5), pp. 520-31.

Pinese, C., Lin, J., Milbreta, U., Li, M., Wang, Y., Leong, K. W. and Chew, S. Y. (2018) 'Sustained delivery of siRNA/mesoporous silica nanoparticle complexes from nanofiber scaffolds for long-term gene silencing', Acta Biomater, 76, pp. 164-177.

Romo, T., McLaughlin, L. A., Levine, J. M. and Sclafani, A. P. (2002) 'Nasal implants: autogenous, semisynthetic, and synthetic', Facial Plast Surg Clin North Am, 10(2), pp. 155-66.

Schallenberger, M. A., Rossmeier, K., Lovick, H. M., Meyer, T. R., Aberman, H. M. and Juda, G. A. (2014) 'Comparison of the osteogenic potential of OsteoSelect demineralized bone matrix putty to NovaBone calcium-phosphosilicate synthetic putty in a cranial defect model', J Craniofac Surg, 25(2), pp. 657-61.

Stenlund, P., Omar, O., Brohede, U., Norgren, S., Norlindh, B., Johansson, A., Lausmaa, J., Thomsen, P. and Palmquist, A. (2015) 'Bone response to a novel Ti-Ta-Nb-Zr alloy', Acta Biomater, 20, pp. 165-175.

Valencia-Lazcano, A. A., Román-Doval, R., De La Cruz-Burelo, E., Millán-Casarrubias, E. J. and Rodríguez-Ortega, A. (2018) 'Enhancing surface properties of breast implants by using electrospun silk fibroin', J Biomed Mater Res B Appl Biomater, 106(5), pp. 1655-1661.

Wang, Q., Qiao, Y., Cheng, M., Jiang, G., He, G., Chen, Y., Zhang, X. and Liu, X. (2016) 'Tantalum implanted entangled porous titanium promotes surface osseointegration and bone ingrowth', Sci Rep, 6, pp. 26248.

Wick, G., Backovic, A., Rabensteiner, E., Plank, N., Schwentner, C. and Sgonc, R. (2010) 'The immunology of fibrosis: innate and adaptive responses', Trends Immunol, 31(3), pp. 110-9.

Tables:

Table 1: Cell viability assessment:

Optical density was used to measure cell viability of fibroblast and osteoblast at a wavelength of 450 nm. For fibroblast, OD of Ta-treated silicone implant materials (0.060 ± 0.013) was higher than that of untreated silicone implant materials (0.054 ± 0.008) ($p = 0.4$). In case of Ta-treated ePTFE, tantalum treated implants showed results (0.089 ± 0.034) that are comparable with untreated implants (0.062 ± 0.023), p-value = 0.4. The result was not statistically significant. In case of osteoblast viability, even though the OD measurements were higher in Ta-treated implant materials compared with untreated materials, the result was not statistically significant.

Group	Mean ± SD Fibroblast	p.value	Mean ± SD Osteoblast	p.value
Ta- treated silicone	0.060 ± 0.013	0.4	0.173 ± 0.101	0.7
Untreated silicone	0.054 ± 0.008		0.128 ± 0.070	
Ta- treated ePTFE	0.089 ± 0.034	0.4	0.349 ± 0.285	0.7
Untreated ePTFE	0.062 ± 0.023		0.202 ± 0.081	

Table 2: Histological evaluation of silicone implant:

Thickness of the soft tissue was expressed by micrometer with mean and SD. In case of 4-weeks term, The thickness of soft tissue in Ta treated implant was 73±34 μm while in untreated group the thickness was 92 ± 43. The difference was not statistically significant. p.value equals 0.7. In 8-week term p.value was 0.3. this result is not statistically significant also. New bone formation in silicone implant material showed similar results in short term, no new bone formation. In 8-weeks term experiment new bone was observed and the mean ± SD was almost the same between Ta- treated and n untreated silicone implant material (0.20 ± 0.45)

	Soft tissue thickness			New bone formation		
	Mean± SD		p.value	Mean± SD		p.value
	Ta. treated	Untreated		Ta. treated	Untreated	
4 weeks	73±34	92 ± 43	0.7	0.00	0.00	-
8 weeks	82±27	115±54	0.3	0.20 ± 0.45	0.20 ± 0.45	1.00

Table 3: Histological evaluation of ePTFE implant:

Soft tissue thickness was expressed in micrometers as the mean ± SD. In case of 2-weeks term, the thickness of soft tissue in the Ta-treated implant was 95 ± 71 μm, while in the untreated group, the thickness was 111 ± 70 μm. The result was better for the Ta-treated group, but the result was not statistically significant (p-value = 0.6). In 4-weeks-term study, the thickness in the Ta-treated implant was 38 ± 17 μm, while in the untreated group, the thickness was 70 ± 63 μm. In addition, the result was better for the Ta-treated group. However, the result was not statistically significant (p-value = 0.7). New bone formation in the ePTFE implant material showed better result in the Ta-treated implant. However, there was no statistical significance observed either in short-term (p-value = 0.6) or in long-term (p-value = 0.6) studies.

Soft tissue thickness				**New bone formation**		
	Mean± SD		p.value	Mean± SD		p.value
	Ta. treated	Non-treated		Ta. treated	Non-treated	
2 weeks	95 ± 71	111 ± 70	0.6	0.60 ± 0.55	0.50 ± 0.58	0.8
4 weeks	38 ± 17	70 ± 63	0.7	1.40 ± 0.89	1.33 ± 1.15	0.9

Figure Legends:

Fig. 1. Intra-operative photograph showing the implant material adapted to the calvarium before suturing. Rat's head was shaved and disinfected prior to making an incision. A 2-cm long transverse incision was made on rat's calvarium. Then, the implant was placed subperiosteally over the calvaria.

Fig. 2.   Transmission and scanning electron microscopy images of the tantalum implanted ePTFE implant. (A) Transmission electron microscopy image: (a) protective carbon deposition was formed around the implant material after coating; (b) tantalum layer after ion implantation; ion implantation was performed using the sputter gun technology; (c) ePTFE implant material. (B) Tantalum layer after ion implantation; ion implantation was performed using the sputter gun technology. The thickness of this layer is approximately 20~30 nm. (C) Scanning electron microscope image of the ePTFE implant material. Long two headed arrow indicates carbon deposition (protective layer). Small two headed arrow indicates platinum coating (protective layer). Single headed arrow indicates tantalum region (another protective layer). (D) ePTFE material.

 Fig. 3. Photomicrograph of histological slides showing biological responses towards Ta-treated and untreated facial implants. (A) Ta-treated silicone implant material (8-week interval). Long two headed arrow indicates soft tissue thickness between the implant material and the bone. (B) Ta- treated silicone implant material (8-week interval). Single headed arrow indicates a dense thick band of fibrous tissue. (C) Ta-treated ePTFE implant (4-week interval). The presence of new bone is observed (indicated by an arrow). (D) Treated ePTFE implant material (4-week interval). The presence of new bone was also observed (newly formed bone is indicated by an arrow). Thin fibrous band is recognized, and single headed arrow is used to indicate it (H & E stain, 400×).
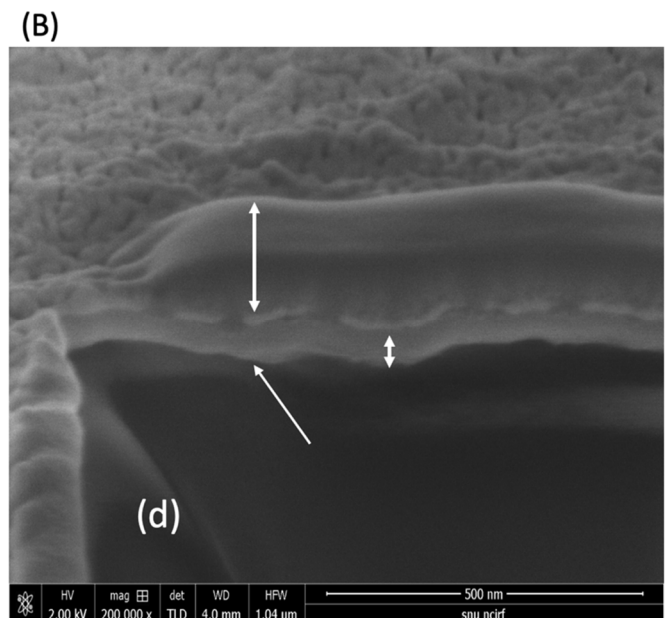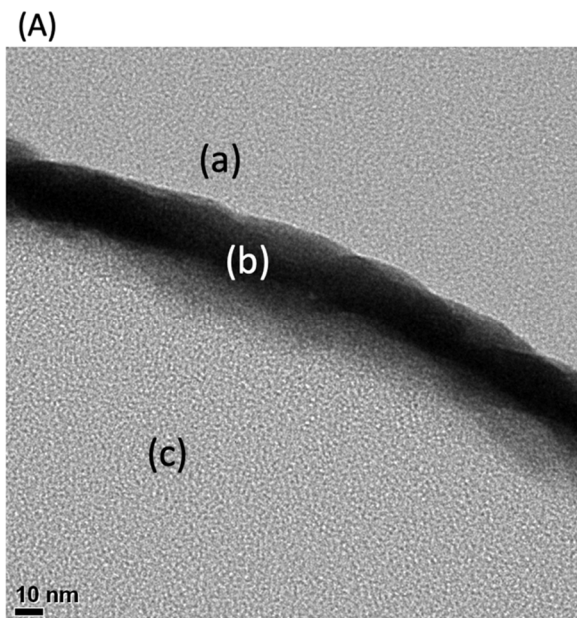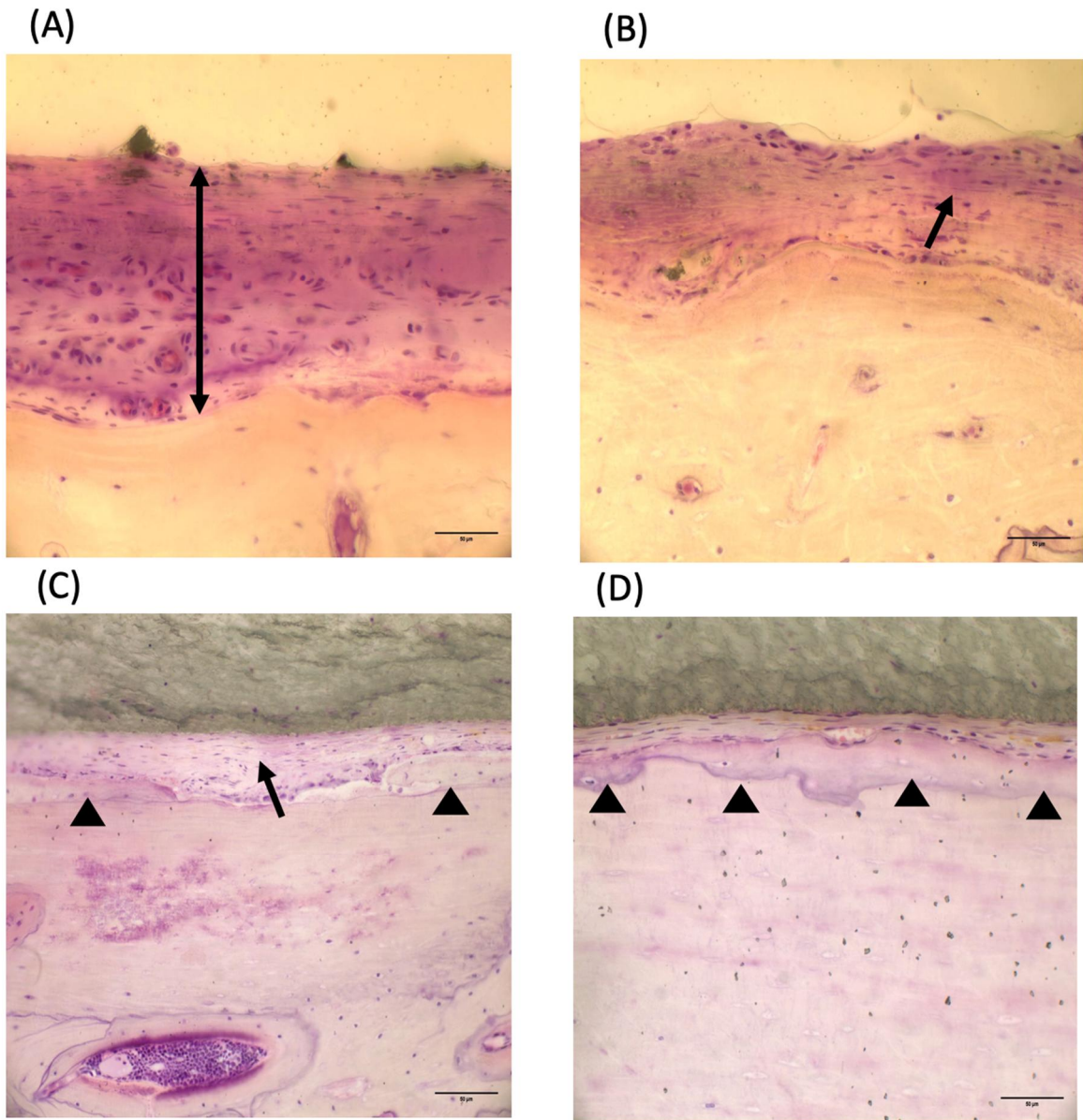
IX. Figures

Fig. 1.

Fig. 2.

Fig. 3.

(A)

(B)

(C)

(D)

탄탈륨 표면 처리에 의한 안면 임플란트 재료의 조직생물학적 기능 향상

서울대학교 대학원 치의과학과 석사과정 구강악안면외과학 전공

모하마드 바크리 (Bakri Mohammed Mousa H)

서울대학교 대학원

치의과학과 구강악안면외과 전공

(지도교수 이 종 호)

배경 : 최근에 임플란트의 생체 적합성을 향상시키기 위해 표면 개질 기술들이 다양하게 연구되고 있는데, 이러한 표면 개질 방법 중에서 생체 특성이 우수한 금속을 고분자 표면에 이온 주입하여 표면 생체 특성을 향상시키는 금속 이온 주입 (metal ion implantation) 기술이 대두되고 있다. 생체 친화형 금속에는 우수한 내부식성과 생체적합성을 보유한 탄탈륨 (Ta)이 있으며 특히 경질 및 연조직 부착을 촉진시키는 뼈 모양의 인회석 (apatite) 형성을 통해 생체 조건 하에서 골 유착 및 성장을 촉진하는 것으로 보고되었다. 본 연구에서는 상용의 안면이식체에 탄탈륨 이온 주입을 통한 표면 개질을 하였을 경우 기존 안면 임플란트 소재의 조직학적 반응에 어떠한 영향을 주는지 확인하고자 하였다.

방법 : 본 연구에서는 안면 이식재로 가장 널리 사용되고 있는 실리콘과 ePTFE 소재에 탄탈륨 이온 주입 표면 개질을 시행하고 표면 개질 후 각 이식재의 연조직 및 골조직 형성 효과를 in-vitro 세포 실험 및 in-vivo 동물 실험을 통해 비교하였다. In-vitro 세포

실험의 경우 섬유아세포와 조골모세포를 이용하여 세포 생존능 변화를 확인하였으며,

in-vivo 동물 실험은 표면 개질 전후의 이식재를 백서 두개골 상방에 매식하여

이식체의 이물반응, 두개골의 골조직 양상과 임식체 주변 연조직의 두께를

조직학적으로 비교분석하였다.

연구결과: 탄탈륨을 이용한 안면 이식재의 표면처리는 통계적으로 유의미한 차이는 아니지만 조골모세포와 섬유아세포의 향상된 증식 효과 경향성을 보였다. 동물실험 결과 탄탈륨 표면 처리 전후 샘플 모두 전체적으로 유사한 이물반응을 확인되었다. 하지만 탄탈륨 표면처리한 샘플군에서 이식재 표면과 두개골 사이에 연조직 형성이 보다 적었고 새로운 골조직이 재생된 점을 보아 탄탈륨 표면처리 후 이식재의 향상된 조직학적 반응을 확인하였다.

결론 : 안면이식재의 탄탈륨 이온 주입은 통계적으로 유의한 수준은 아니나 섬유모세포와 골모세포의 생존능을 증가시키는 경향을 나타냈다. 동물실험에서 탄탈륨 이온 주입을 한 이식체나 비처리 이식재 모두에서 유사한 이물반응을 보였다. 하지만 임플란트와 하부 두개골 사이에 연조직 개재량이 적었으며, 또한 더 많은 신생골 형성능을 나타내 더 나은 조직 반응을 보여 주었다. 이러한 연구 결과는 생체 특성이 우수한 탄탈륨이 고분자 안면 이색재의 표면 개질 소재로 활용될 수 있는 가능성을 시사하였다.

주요 단어 : 탄탈륨이온주입, 표면처리, 안면이식재, ePTFE, 실리콘

학번 : 2017-29267.