Original Article

Effect of different sterilization modes on the surface morphology, ion release, and bone reaction of retrieved micro-implants

Noha El-Wassefy[a]; Abeer El-Fallal[b]; Mahasen Taha[c]

ABSTRACT

Objective: To compare as-received and sterilized micro-implants in order to assess the prospects of reusing them.

Materials and Methods: Forty micro-implants from a single manufacturing lot were used in the study. Thirty were retrieved from patients after successful service in their mouth and with no signs of failure. The retrieved micro-implants were divided into three groups, according to method of sterilization: autoclave, gamma radiation, or ultraviolet radiation. All groups were subjected to scanning electron microscope analysis for surface morphology assessment. The specimens were immersed in a standard simulated body-fluid solution kept at 37°C in an incubator; the solution was then withdrawn at 24 hours and 30 days to evaluate aluminum and vanadium ion release by atomic absorption spectrophotometer in parts per billion. The micro-implants were then surgically implanted into the tibia of rabbits for a 1-month healing period, and the bone-implant blocks were processed for routine histologic examination.

Results: This study revealed that sterilized micro-implants had altered surface topography, different ion release values, and different histologic cell reactions than the as-received micro-implants.

Conclusions: Within the limitations of this study, it can be concluded that retrieved self-drilling micro-implants have tip sharpness variations that require correction before insertion by bone drilling. The autoclave-sterilized micro-implants showed better histologic results than micro-implants sterilized by gamma or ultraviolet rays. (*Angle Orthod.* 2015;85:39–47.)

KEY WORDS: Micro-implants; Sterilizations; Surface morphology; Ion release; Bone interface histology

INTRODUCTION

Micro-implants are used in orthodontics as a skeletal anchorage system. They are popular because they have a wide variety of applications, require less traumatic surgery, and present the possibility of immediate loading.[1–7] Ethical considerations about reuse of implants in different patients exist, but they can be reused in the same patient.[8] When relocation of a micro-implant to another position is planned, it is only possible if their structural integrity and mechanical properties are not altered after their prior use and sterilization.[9]

Commonly used sterilization processes are steam autoclaving and gamma irradiation.[10,11] Another promising method is ultraviolet light.[12] However, sterilization may contribute to changes in surface topography and mechanical resistance of the mini-implant; thus, this study compares as-received mini-implants under three methods of sterilization: autoclave, gamma rays, and ultraviolet light to answer the question of whether mini-implants can be reused.

MATERIALS AND METHODS

The 40 micro-implants used in this study were from the same manufacturer (AbsoAnchor Micro-implant GH 1413-07, Lot MI80328C, Dentos, Daegu, Korea). The micro-implants were self-drilling, were made from Ti6Al4V alloy, and had a diameter of 1.4 mm and a thread length of 7 mm.

[a] Assistant Professor of Dental Biomaterials, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.

[b] Associate Professor of Dental Biomaterials, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.

[c] Associate Professor of Orthodontics, Faculty of Dentistry, Mansoura University, Mansoura, Egypt.

Corresponding author: Dr Noha El-Wassefy, Mansoura University, Faculty of Dentistry, Mansoura 35516 Egypt (e-mail: nohahmed@mans.edu.eg)

Accepted: April 2014. Submitted: January 2014.
Published Online: May 27, 2014

Thirty micro-implants were retrieved from patients after successful service of 5 to 18 months and no signs of failure, such as peri-implant soft-tissue inflammation, implant mobility, or premature loss. The micro-implants were removed by applying a counterclockwise torqueing load with a specially designed driver provided by the manufacturer. After removal, each micro-implant was stored, completely immersed in distilled water, in a sterile container used for lab assessments.

The micro-implants were divided into four groups, according to their condition: as-received (control), autoclaved, gamma sterilized, and ultraviolet sterilized.[13] Ten mini-implants were inserted in an individual auto-sealing envelope and then submitted to one sterilizing cycle of 30 minutes at 121°C and 18 psi, according to the recommendations of the manufacturer of the autoclave used (Dental autoclave sterilizer model no. DA-03: 23L; GaoDin Medical Co, Ltd, Shanghai, China). Ten mini-implants were sterilized by gamma radiation in a gamma sterilizer at 25 kGy overnight, after insertion in gamma sterilization pouches. Ten mini-implants were sterilized by ultraviolet radiation via ultraviolet light for 90 minute at 254 nm (UV cross-linker; CL-1000; Thermo Fisher Scientific Inc, Waltham, Mass). The third set of 10 micro-implants served as a control group and were analyzed and tested as received from the manufacturer.

Surface Characterization

Surface morphology was assessed by scanning electron microscopy (SEM; JXA-480A electron probe microanalyzer, JEOL, Tokyo, Japan) at an operating voltage of 30 kV.

Metal Ion Release

Metal ion release tests were performed by immersing the samples in a standard simulated body fluid (SBF) solution kept at 37°C in an incubator. The SBF used in this work was proposed by Kokubo and coworkers[14] to mimic the inorganic salt composition of human physiological fluids. Half of the solution was withdrawn at 24 hours and 30 days, respectively, and replaced with fresh solution. The concentrations of aluminum and vanadium ions released from the Ti6Al4V micro-implants into SBF were measured using a graphite furnace atomic absorption spectrophotometer (model TAS990, Intec Co Ltd, Rome, Italy).

Surgical Procedure

Animals. Four male, 6-month-old New Zealand white rabbits weighing between 3.0 and 3.5 kg, were used in the research. The surgical procedure consisted of implanting two micro-implants on each side separated with a 1 cm distance into the right and left tibial metaphysis of each animal. The current study protocol was approved by the committee of animal research and was conducted at the Mansoura Experimental Research Center, Faculty of Medicine, Mansoura University. All surgeries were performed under sterile conditions in a veterinary operating room.

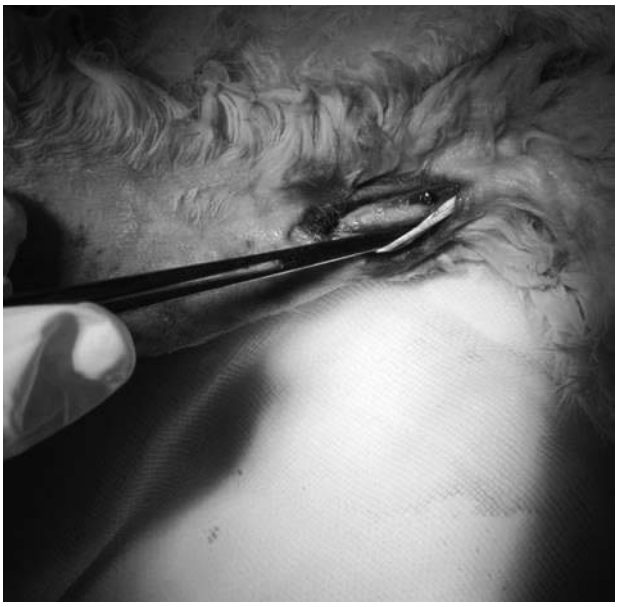

Figure 1. Rabbit tibia with self-drilling micro-implant in situ.

Surgical insertion. The animals were anesthetized with an intramuscular injection of ketamine 50 mg/kg body weight (ketamine as HCl; BN 1002381, EIPICO, Ramadan, Egypt), and 50 mg/mL xylazine was administered intramuscularly (xylazine 20 mg M.H. Reg No. 1373/2009 Vet, ADWIA, El-Oubor City, Egypt). Immediately before surgery, 1.0 mL of local anesthetic solution was injected at the surgical area (mepivacaine HCl 2% Mepecaine-L, BN 2412152 Alexandria Co. for Pharmaceuticals, Alexandria, Egypt). Hair on the legs was shaved, and the skin was cleansed with iodine. A 20-mm incision was made parallel to the longitudinal axis of the tibia, and the periosteum was stripped, denuding the bone. The implantation holes were drilled under profuse saline irrigation, using a drill diameter of 1.0 mm, operating at low rotatory speed (500 rpm). The micro-implant was threaded at the cortex of the tibia (Figure 1) using a holder key; subsequently, the soft tissues were closed in layers with absorbable sutures. Antibiotics (cephalosporin, 1 g/day for 5 days) and analgesics (ketoprofen 500 mg/day for 3 days) were administered postoperatively.[15]

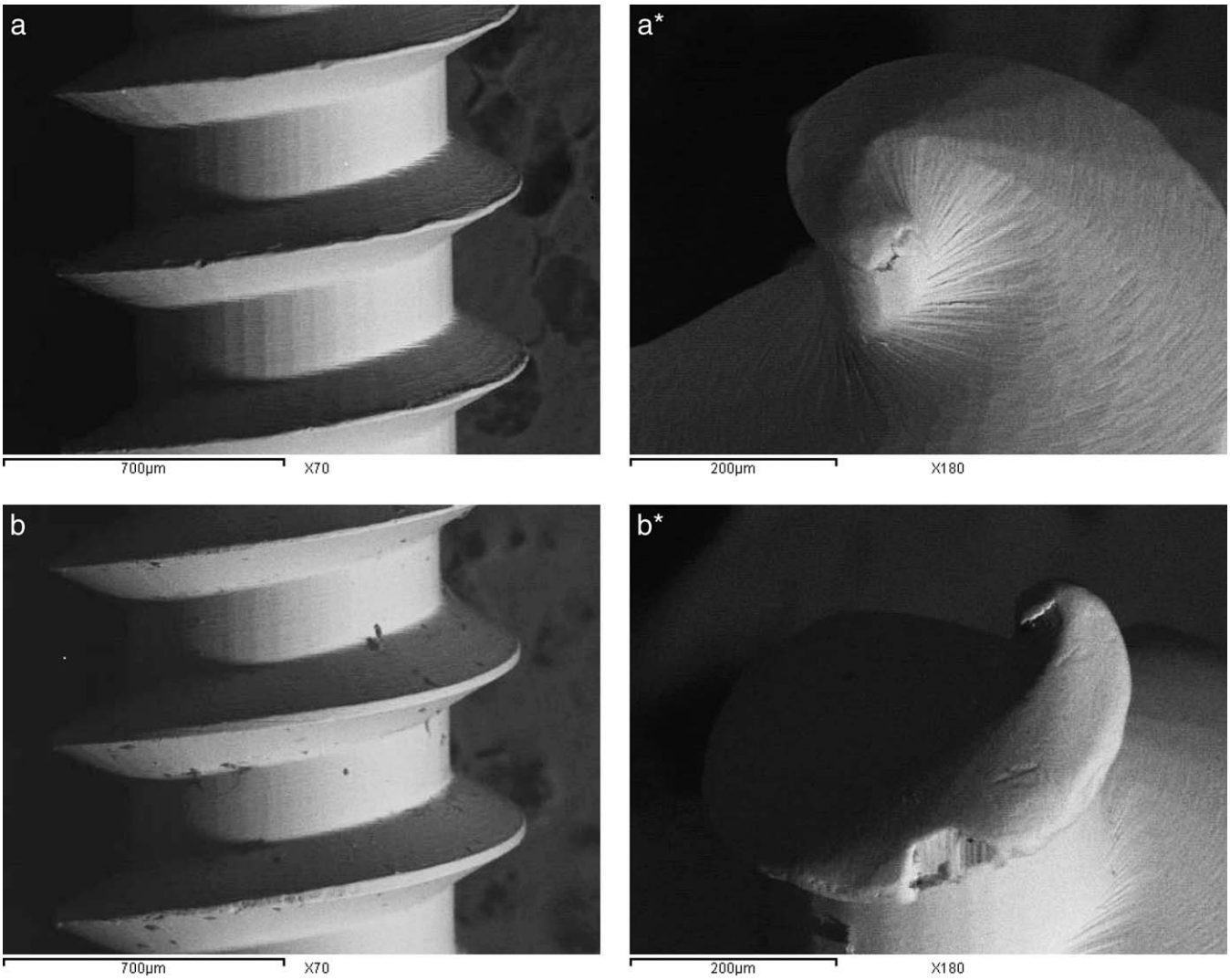

Figure 2. (a) SEM at magnification ×70 showing the flutes of the control as-received micro-implant. (a*) SEM at magnification ×180 showing the tip of the same micro-implant. (b) SEM at magnification ×70 showing the flutes of a retrieved micro-implant sterilized by autoclave. (b*) SEM at magnification ×180 showing the tip of the same micro-implant.

Histologic Assessment

After a 1-month healing period, the animals were killed by a massive dose of thiopental, the tibiae were dissected, and blocks containing one micro-implant and at least 2 mm of surrounding bone were sectioned and fixed in ethylenediaminetetra-acetic acid and then processed for routine histologic evaluation.

Statistical Analysis

For significance of differences, the data were evaluated by the Kruskal-Wallis nonparametric test. Pairwise comparisons were performed to test the significance between the different treatment methods and the control sample. The significance limit was predetermined in the confidence interval of 5%.

RESULTS

Surface Morphology Using SEM

Figure 2 shows the surface morphology at magnifications ×70 and ×180 of as-received micro-implants (Figure 2a,a*) and micro-implants sterilized by autoclave (Figure 2b,b*), by gamma rays (Figure 2c,c*), and by ultraviolet radiation (Figure 2d,d*) visualized by SEM. The photomicrographs show noticeable grooves in the flutes of micro-implants due to the machining procedure. No defects in the form of pores or cracks and no image indicative of corrosion could be identified in the flutes of the sterilized micro-implants or the as-received micro-implants. On the other hand, the retrieved micro-implants showed a smoother surface on the threads and scratch marks on the tip compared with the as-received micro-implants.

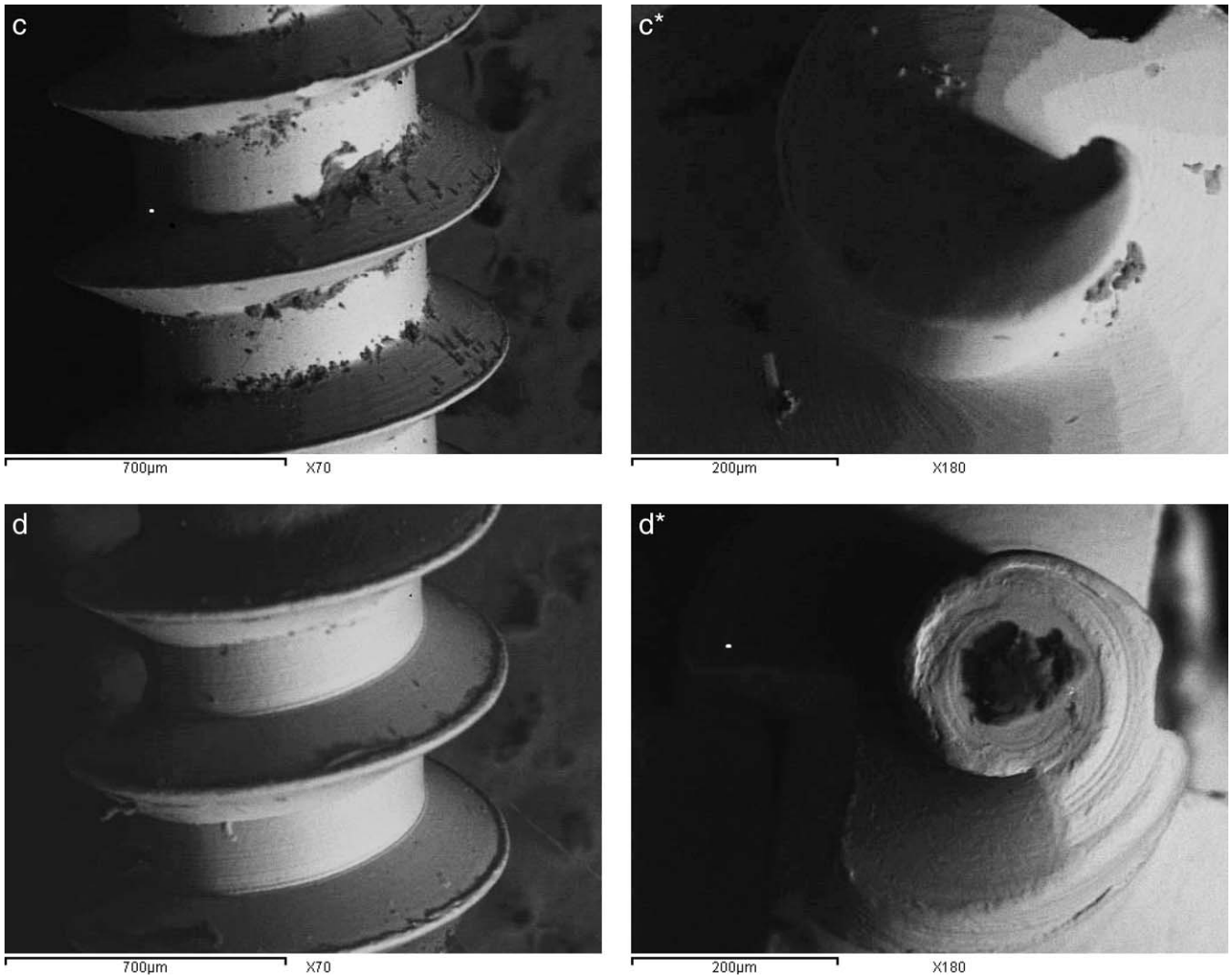

Figure 2. (c) SEM at magnification ×70 showing the flutes of a retrieved micro-implant sterilized by gamma radiation. (c*) SEM at magnification ×180 showing the tip of the same micro-implant. (d) SEM at magnification ×70 showing the flutes of a retrieved micro-implant sterilized by ultraviolet radiation. (d*) SEM at magnification ×180 showing the tip of the same micro-implant. SEM indicates scanning electron microscopy.

The photomicrographs at magnification ×180 show the blunt tips with smoother edges of retrieved mini-implants (Figure 2b*,c*,d*), whereas the as-received micro-implant (Figure 2a*) shows a sharp, well-defined, scratch-free tip.

The gamma ray–sterilized micro-implants show irregularly distributed rough surface deposits visible at both magnifications (Figure 2c,c*). For the micro-implants sterilized by ultraviolet radiation, the surface deposits were fewer in quantity and more related to the outer border of the flutes (Figure 2d,d*).

Metal Ion Release

The results are expressed as the average of triplicate determinations ± the standard deviation, as presented in Table 1. The data were evaluated by the Kruskall-Wallis nonparametric test. Pairwise comparisons were performed to test the significance among

Table 1. Means and SDs for Aluminum and Vanadium Ion Release after 1 day of Immersion in Simulated Body Fluid[a]

	n	Aluminum Mean (ppb)	Aluminum SD (ppb)	Vanadium Mean (ppb)	Vanadium SD (ppb)
Control	5	98.2880	2.34187	48.1220	1.39954
Autoclave sterilized	5	22.3820*	2.06150	47.5100	0.69387
Gamma ray sterilized	5	27.5120	2.52259	66.5420*	1.06851
Ultraviolet sterilized	5	14.5560*	2.69760	61.4620	1.14740

[a] Values with * indicate significant difference from the control (Kruskal-Wallis $P < .05$); ppb indicates parts per billion; SD, standard deviation.

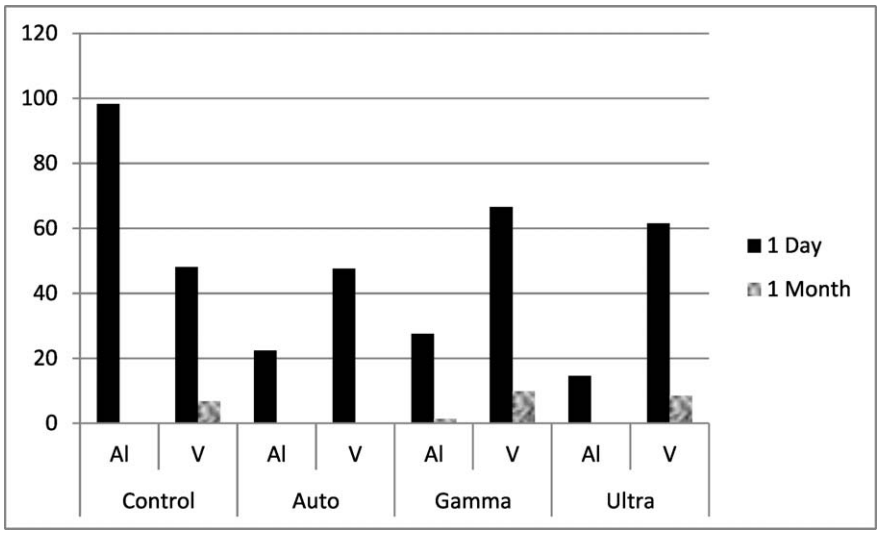

Figure 3. Aluminum and vanadium concentrations (in parts per billion) for the control as-received micro-implants and for the micro-implants sterilized by autoclave, gamma-rays, and ultraviolet radiation at the 1-day and 1-month immersion periods.

the different treatment methods and the control sample.

In this study, aluminum ion was detected in the SBF at incubation time of 1 day for the as-received and sterilized micro-implants; the detected aluminum ion released from the control micro-implant (the as-received group) in SBF at 1 day was significantly higher than for the other sterilized groups. The ultraviolet-sterilized micro-implants released the lowest level of aluminum ion into the SBF. On the other hand, the detected vanadium ion released from the as-received and autoclave-sterilized micro-implants in SBF at 1 day were significantly lower than for the micro-implants sterilized by gamma rays.

After 1 month of immersion in SBF, all groups had a comparable significant decrease in aluminum and vanadium ion released in SBF, which was undetected in several samples (Figure 3).

Surgical Procedure Results: Macroscopic Examination of Implant–Bone Interface

After a 1-month healing period, scarification and exposure of the implantation area was done. It was found that all micro-implants were steady, and when probed with a pair of forceps, a good mechanical fixation was noticed for micro-implants in all groups. No signs of inflammation or adverse tissue reaction were observed.

Histologic Examination of the Bone–Implant Interface

Representative microscopic sections of bone–implant interface are shown in Figure 4. The photomicrograph of the as-received control micro-implant (Figure 4a,a*), shows the empty space previously occupied by the implant bordered by a lamellate bone of low cellular activity and well-arranged bone trabeculae; neither inflammatory cell infiltrate nor infection signs were observed.

A photomicrograph of the autoclave-sterilized group (Figure 4b,b*) shows the empty space previously occupied by the implant bordered by a woven bone of high cellular activity and irregularly arranged bone trabeculae. Osseous bridges between trabecular bone are clearly visible, and there are abundant osteoblast-like cells on the endocortical bone surface.

A photomicrograph of the gamma ray–sterilized group (Figure 4c,c*) shows the empty space previously occupied by the implant bordered by a granulation tissue with inflammatory cell, fibroblast proliferation, and the beginning of osteoid tissue deposition. There is fibrous encapsulation with high vascularity and cellularity, and the bone density appears to be qualitatively of lower value than the other groups.

A photomicrograph of the group sterilized by ultraviolet rays (Figure d,d*), shows the empty space previously occupied by the implant bordered by bone of low cellular activity and well-arranged bone trabeculae.

DISCUSSION

SEM analysis indicated that the processes of sterilization did not alter the micro-implant surface topography; however, their surfaces were smoother, their tips were less sharp, and they had some abrasion marks signifying that the insertion and removal may have resulted in wear. The micro-implant type studied was self-drilling, so the tip alteration may possibly modify its drilling properties, and if it is to be reused, it

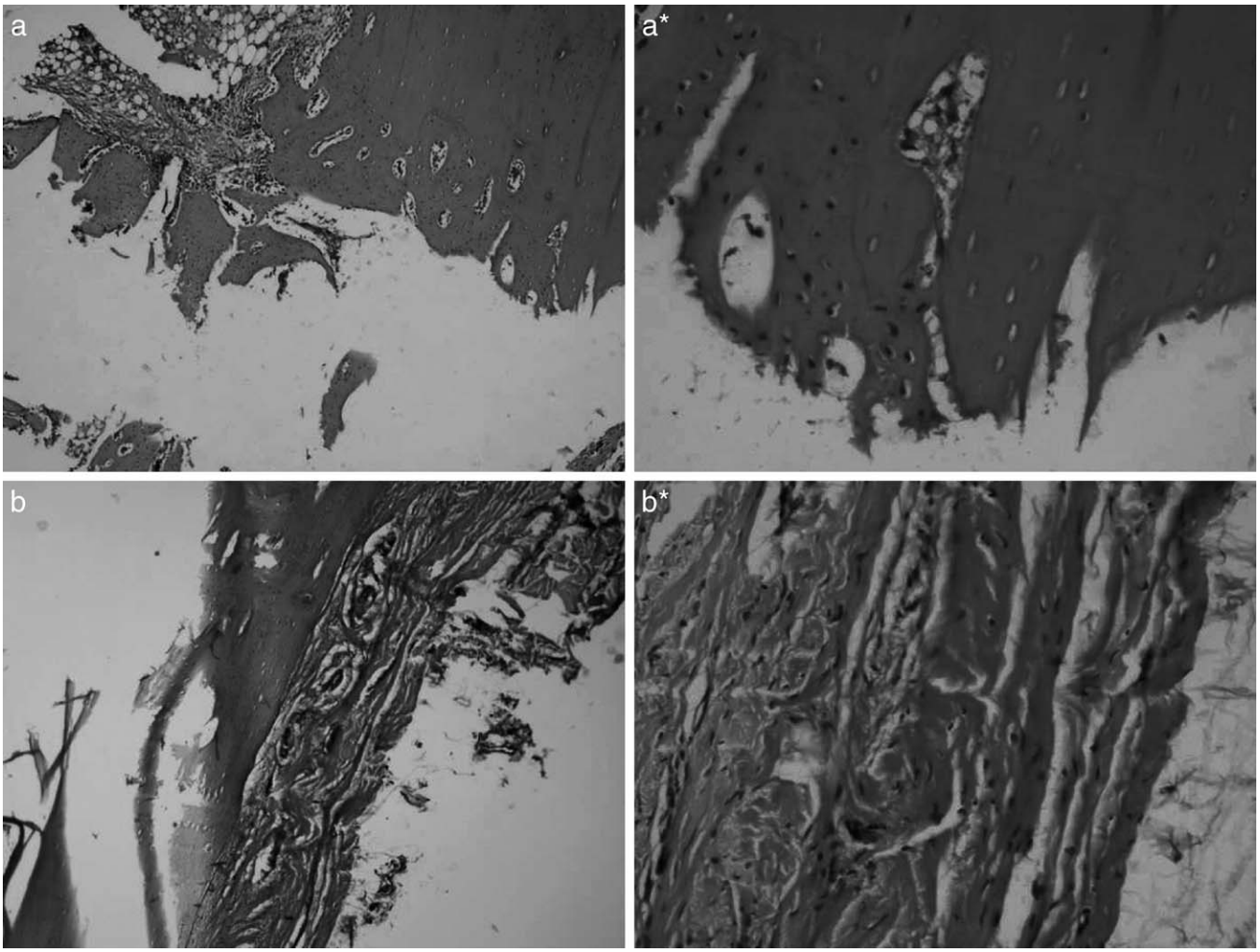

Figure 4. (a) Photomicrograph of the control as-received group showing the empty space previously occupied by the implant bordered by lamellar bone of low cellular activity and well-arranged bone trabeculae (H&E ×100). (a*) Photomicrograph of the control as-received group (H&E ×400). (b) Photomicrograph of the autoclave-sterilized group showing the empty space previously occupied by the implant bordered by woven bone of high cellular activity and irregularly arranged bone trabeculae (H&E ×100). (b*) Photomicrograph of the autoclave-sterilized group (H&E ×400).

may require bone drilling before insertion. Eliades et al.[16] found morphologic and superficial structural changes in retrieved micro-implants but no material structural alterations in the form of defects or pores. Schwartz et al.[17] also detected deep scratch marks on the surface of used cover-screws.

The gamma-sterilized (and to a lesser extent the ultraviolet sterilized) micro-implants showed irregularly distributed rough surface deposits that seemed to be formerly deposited bone that is sustained mainly after sterilization with gamma rays, as gamma rays are usually used to sterilize connective tissue allografts, such as bone in tissue banking. It is hypothesized that polypeptide chain scissions prevail when collagen is irradiated in a dry state because of the direct effect of ionizing radiation; however, a cross-'linking reaction occurs during the collagen irradiation in existence of water.[18]

Changes in surface morphology due to cleaning and/or mechanical damage during placement and removal can result in marked alterations in osteoblastic growth and differentiation.[17] Furthermore, cell attachment levels may be lower and cell spreading reduced in titanium autoclaved surfaces.[12] For that reason, reuse may not be considered for micro-implants that rely on osseointegration for stability but only for those designed to become stable by mechanical interdigitation to the bone.

Titanium exhibits excellent biocompatibility and belongs to the loose connective vascularized group with regards to tissue reaction.[19] The effect of aluminum concentration on cell viability depends on surface roughness, surface treatment, and strength of the oxide film. Vanadium has acute and chronic toxic effects when absorbed in greater amounts.[20] It can

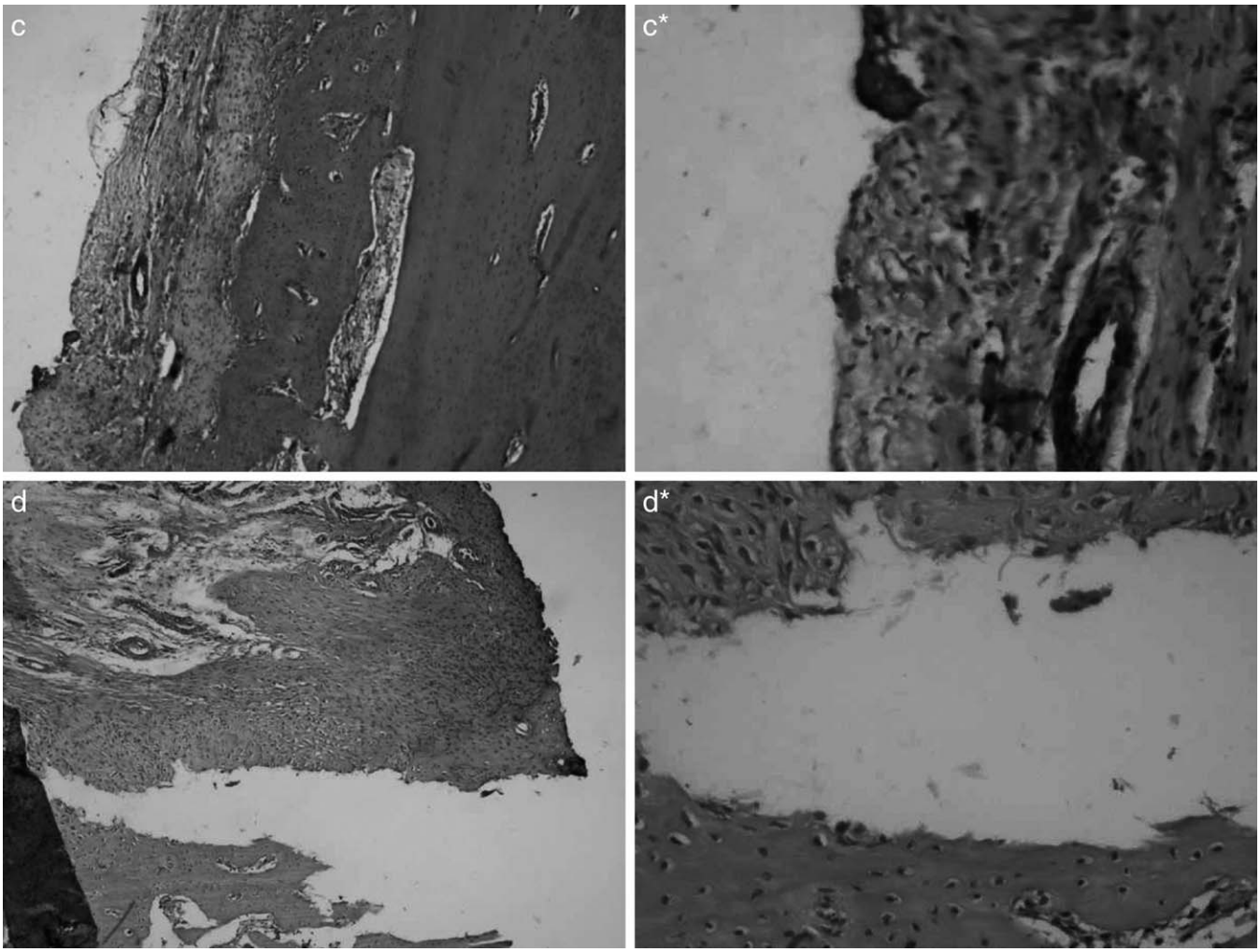

Figure 4. (c) Photomicrograph of the gamma ray–sterilized group, showing the empty space previously occupied by the implant bordered by a granulation tissue and fibrous encapsulation with high vascularity and cellularity (H&E ×100). (c*) Photomicrograph of the gamma ray–sterilized group (H&E ×400). (d) Photomicrograph of the ultraviolet-sterilized group showing the empty space previously occupied by the implant bordered by a granulation tissue with inflammatory cell, fibroblasts proliferation, and the beginning of osteoid tissue deposition (H&E ×100). (d*) Photomicrograph of the ultraviolet-sterilized group (H&E ×400). H&E indicates hematoxylin and eosin.

arouse local and systemic reactions and hinder cellular proliferation.[21] According to the theory of passivity, metallic biomaterials in aqueous solutions are systems in which active and passive surfaces exist simultaneously in contact with electrolyte. Therefore, it is now thought that the surface oxide film on the materials repeats a process of partial dissolution and precipitation in aqueous solution. In Ti6Al4V, titanium, aluminum, and vanadium are released in SBF.[22]

In this study, the detected aluminum ion released from the as-received micro-implant in SBF at 1 day was significantly higher than that of the other sterilized groups. On the other hand, the detected vanadium ion released from the as-received and autoclaved micro-implants in SBF at 1 day were significantly lower than amount released by the micro-implants sterilized by gamma rays or ultraviolet rays. The surface of as-received micro-implant has an undisturbed titanium oxide surface film that allow for more ion exchange than the other retrieved micro-implants, which that had a more mature titanium oxide film. In aluminum-containing alloys, a significant aluminum ion release was registered because of a great driving force for ion migration combined with smaller, more mobile aluminum ions.[23]

Calcium ions are adsorbed by phosphate ions adsorbing on a hydrated titanium surface, and eventually calcium phosphate is formed. The calcium to phosphorus ratio increases with increasing time of immersion.[24]

After the micro-implants were immersed in SBF for 1 month, aluminum, and vanadium ions were undetected in SBF and showed a significant decrease compared with 1 day of immersion; this might be

attributed to the precipitation of calcium/phosphorus minerals from the SBF into the titanium surface, which decreases the ion release.

The bone ingrowth into rough surfaces of the titanium implants creates a steady, retentive interface between the implant and the preexisting bone.[25] Chang and Kao[26] investigated the biomechanical behavior and histology of particulate hydroxyapatite implanted in bone defects created in dogs and showed a consistent increase in compressive stress and shear stress at each stage that was responsive to the ossification and maturation of the ingrowing tissue within the bone defect.[26]

The qualitative observations of the light microscopically stained sections revealed a higher amount of endosteal new bone tissue formation along the micro-implant surface. Regarding the gamma-sterilized dental micro-implants, bone deposits were noticed via SEM (Figure 2c) and caused inflammatory reactions after implantation in the rabbit tibia as shown in the histologic sections (Figure 4c); this may be attributed to the rabbit body recognizing the remaining human bone as foreign material after being retrieved and sterilized by gamma rays. However, the remnant bone might help speed up osseointegration, as the body recognizes its own bone cells deposited on the micro-implant surface when relocating after sterilization. Ueno et al.[27] concluded that gamma ray–treated titanium implants may potentially enhance osteoconductivity and can overcome the biological aging of titanium.[27]

CONCLUSIONS

Within the limitations of this study:

- Whatever the method of sterilization is used, the micro-implant surface cannot be considered the same as the original in terms of surface morphologic properties, ion release, and histologic cell response.
- The autoclave-sterilized micro-implants showed better histologic results than the micro-implants sterilized by gamma rays or ultraviolet rays.

ACKNOWLEDGEMENTS

The authors acknowledge Dr Samah El-Sheikh, veterinarian, from the Mansoura Experimental Research Center, for her assistance and care through all the surgical procedures. We also thank Dr Mohamed El-Awady, professor of oral biology, Faculty of Dentistry, Mansoura University, for interpreting the histologic sections.

REFERENCES

1. Kanomi R. Mini-implant for orthodontic anchorage. *J Clin Orthod*. 1997;31:763–767.
2. Freudenthaler JW, Haas R, Bantleon HP. Bicortical titanium screws for critical orthodontic anchorage in the mandible: a preliminary report on clinical applications. *Clin Oral Implants Res*. 2001;12:358–363.
3. Bae SM, Park HS, Kyung HM, Kwon OW, Sung JH. Clinical application of micro-implant anchorage. *J Clin Orthod*. 2002; 36:298–302.
4. Park HS, Kyung HM, Sung JH. A simple method of molar uprighting with micro-implant anchorage. *J Clin Orthod*. 2002;36:592–596.
5. Kyung HM, Park HS, Bae SM, Sung JH, Kim IB. Development of orthodontic micro-implants for intraoral anchorage. *J Clin Orthod*. 2003;37:321–328; quiz 314.
6. Herman RJ, Currier GF, Miyake A. Mini-implant anchorage for maxillary canine retraction: a pilot study. *Am J Orthod Dentofacial Orthop*. 2006;130:228–235.
7. Kim TW, Kim H, Lee SJ. Correction of deep overbite and gummy smile by using a mini-implant with a segmented wire in a growing Class II division 2 patient. *Am J Orthod Dentofacial Orthop*. 2006;130:676–685.
8. Karch M, Alt E, Schmitt C, Schomig A. Reimplantation of an infected, abdominally implanted defibrillator in the subpectoral region. *J Cardiovasc Surg (Torino)*. 1996;37:67–70.
9. Carano A, Velo S, Leone P, Siciliani G. Clinical applications of the Miniscrew Anchorage System. *J Clin Orthod*. 2005;39: 9–24; quiz 29–30.
10. Sylvia VL, Boyan BD, Dean DD, Schwartz Z. The membrane effects of 17beta-estradiol on chondrocyte phenotypic expression are mediated by activation of protein kinase C through phospholipase C and G-proteins. *J Steroid Biochem Mol Biol*. 2000;73:211–224.
11. Serro AP, Saramago B. Influence of sterilization on the mineralization of titanium implants induced by incubation in various biological model fluids. *Biomaterials*. 2003;24: 4749–4760.
12. Vezeau PJ, Koorbusch GF, Draughn RA, Keller JC. Effects of multiple sterilization on surface characteristics and in vitro biologic responses to titanium. J Oral Maxillofac Surg. 1996; 54:738–746.
13. Park JH, Olivares-Navarrete R, Baier RE, et al. Effect of cleaning and sterilization on titanium implant surface properties and cellular response. *Acta Biomater*. 2012;8: 1966–1975.
14. Kokubo T, Takadama H. How useful is SBF in predicting in vivo bone bioactivity? *Biomaterials*. 2006;27:2907–2915.
15. El-wassefy NA, Hammouda IM, Habib AN, El-awady GY, Marzook HA. Assessment of anodized titanium implants bioactivity. *Clin Oral Implants Res*. 2014;25:e1–9.
16. Eliades T, Zinelis S, Papadopoulos MA, Eliades G. Characterization of retrieved orthodontic miniscrew implants. *Am J Orthod Dentofacial Orthop*. 2009;135:10 e11–e17; discussion 10–11.
17. Schwartz Z, Lohmann CH, Blau G, et al. Re-use of implant coverscrews changes their surface properties but not clinical outcome. *Clin Oral Implants Res*. 2000;11: 183–194.
18. Kátia Aparecida da Silva Aquino (2012). Sterilization by Gamma Irradiation, Gamma Radiation, Prof. FerizAdrovic (Ed.), ISBN: 978-953-51-0316-5, InTech, Available from: http://www.intechopen.com/books/gamma-radiation/sterilization-by-gamma-irradiation
19. Steinemann SG. Corrosion of surgical implants-in Vivo and in Vitro tests. In: Evaluation of Biomaterials; Winter GD, Leray JL, de Groot K. Eds. Chichester, UK: Wiley. 1980; pp. 1–34.

20. Heinemann G, Fichtl B, Vogt W. Pharmacokinetics of vanadium in humans after intravenous administration of a vanadium containing albumin solution. *Br J Clin Pharmacol*. 2003;55:241–245.
21. Sedarat C, Harmand MF, Naji A, Nowzari H. In vitro kinetic evaluation of titanium alloy biodegradation. *J Periodontal Res*. 2001;36:269–274.
22. Bruneel N, Helsen JA. In vitro simulation of biocompatibility of Ti-Al-V. *J Biomed Mater Res*. 1988;22:203–214.
23. Browne M, Gregson PJ. Surface modification of titanium alloy implants. *Biomaterials*. 1994;15:894–898.
24. Hiromoto S, Hanawa T, Asami K. Composition of surface oxide film of titanium with culturing murine fibroblasts L929. *Biomaterials*. 2004;25:979–986.
25. Kienapfel H, Sprey C, Wilke A, Griss P. Implant fixation by bone ingrowth. *J Arthroplasty*. 1999;14:355–368.
26. Chang RC, Kao AS. Biomechanical and histological studies of particulate hydroxylapatite implanted in femur bone defects of adult dogs. *Int J Oral Maxillofac Surg*. 2000;29:54–61.
27. Ueno T, Takeuchi M, Hori N, et al. Gamma ray treatment enhances bioactivity and osseointegration capability of titanium. *J Biomed Mater Res B Appl Biomater*. 2012;100:2279–2287.