

ABSTRACT

Our line of research tries to rescue out of-date dental implants and return them for suitable patient placement. The two main goals are re-sterilization, and optimization of surface properties. Expired dental implants were divided into three groups of 9 implants each. The implants differed in terms of surface roughness. Two groups were Hydrogen Peroxide Gas Plasma (H₂O₂ GP) treated MK-II [smooth surfaced implants], and MK-III TiUnite [rough surfaced implants]. Third group was as received MK-II samples. The samples were tested for sterility and also test by MTT assay after cell culture with Human Osteoblast. Furthermore, surface analysis by Scanning Electron Microscopy (SEM) and Energy Dispersive X-Ray Spectroscopy (EDS) were conducted. Results showed that implants containing wells were higher in terms of cells' activity than control cell group. Also, sterilization testing revealed that implants were sterile after (H₂O₂ GP) treatment. In Conclusion our results showed that out-of-date dental implants allow for active cells' growth and proliferation and, at the same time, the broth culture media did not show any bacterial growth.

INTRODUCTION

Commercially Pure Titanium (cpTi) dental implant placement become common activity in the dental clinics. In spite of increasing demand for implantation in patients, many dental implants pass their expiration dates on the bench before reaching their intended use. The main challenge is to select the suitable sterilization modality that do not affect implant's surface properties (1,3).

MATERIALS and METHODS

Materials: used in this study were 26 implants from NobelBiocare. However, they were either MK-II [smooth surface] or MK-III TiUnite [rough surface] implants. They were treated as follows: 1)MK-II Gas Plasma in H₂O₂ for 25 minutes for the whole cycle, 2)MK-III TiUnite Gas Plasma in H₂O₂, and 3)As received MK-II samples.

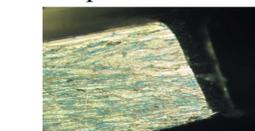
Hydrogen Peroxide Gas Plasma: 50% Hydrogen peroxide liquid introduced into Harrick device to produce H₂O₂ vapor. STERRAD® [J&J, Advanced Sterilizations Products (ASP), FDA approved technique] quarter sterilization cycle was followed in this treatment modality.

Sterilization tests: Implants in BHI broth media were incubated for at least 24 hrs in 37 C and results were obtained by visual inspection and confirmed by agar plate cultures.

MTT Assay: results were collected after 72 hrs of incubation of cells.

SEM and EDS analysis: SEM pictures after cell fixation and dehydration collected, and also EDS spectra were recorded for each samples.

Differential Interference Contrast Light Microscopy: pictures also obtained after cell fixation and dehydration steps.



Differential Interference Contrast Microscopy picture of MK-II surface.



SEM device (4)



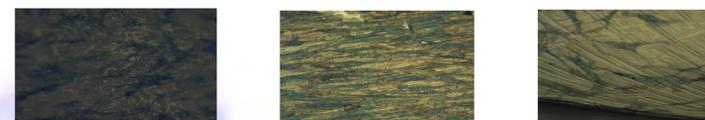
Harrick GP device(4).

RESULTS

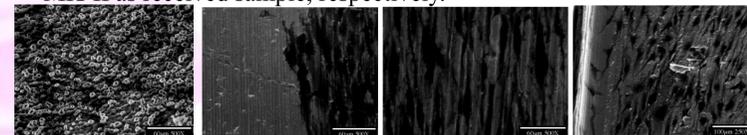
MTT Assay- H₂O₂ GP treated groups [smooth and rough surface] and as received were higher in terms of cells' activity than control cell group.

Sterilization test- H₂O₂ GP treated groups did not show any bacterial growth; however, as received implants did remain also sterile. (not all implants were tested for their sterility)

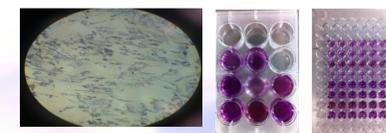
SEM & EDS Analysis: It shows that MK-II implant surface is cpTi surface and apparently smooth, while MK-III TiUnite surface is rough and contain phosphorus due to the acid etching step during manufacturing steps.



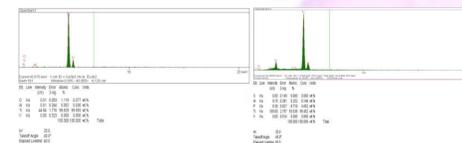
Pics 1-3 Differential Interference Contrast Light Microscopy was used to collect these pictures under 50x magnification power. Pictures are MK-III TiUnite, MK-II Treated surface, and MK-II as received sample, respectively.



Pics (7-10) Pictures obtained from SEM analysis. From left to right pictures are MK-III TiUnite surface, MK-II as received surface, the last two are MK-II treated surface at different magnification power.



Pics (4-6): Pic (4) represents the step of absorbing MTT dye by the living cells. Pics (5) and (6) show the color of the solution after adding the cell lysing agent.



Spectrum of MK-II smooth surface implant. It shows that the implant is basically commercially pure titanium. Left is spectrum of MK-III TiUnite surface, in which phosphorus peak is shown.

CONCLUSIONS

Within the limitation of this study, the results indicate that out-of-date dental implants are showing higher cells' activity than control cell group. Also, hydrogen peroxide gas plasma treatment modality is a suitable technique for implant sterilization.

The sterilization test indicates that GP treatment in H₂O₂ did not allow any bacterial growth, neither in the BHI broth media culture nor in the confirming agar plates. Even though, positive control contaminated samples with *Candida Albicans* were tested; they did not grow any bacteria in the BHI. Interestingly, cells' activity neither showed any differences due to surface treatment treated, nor the roughness of the implant surfaces [MK-III TiUnite] indicated higher cell activity. Moreover, expired-in-package dental implants appear to be sterile even after 10 years of their expiration dates.

REFERENCES

- (1) Baier RE, Meyer AE, Akers CK, Natiella JR, Meenaghan M, Carter JM. Degradative effects of conventional steam sterilization on biomaterial surfaces. *Biomaterials* 1982;3:241-245.
- (2) Rogez-Kreuz C, Yousfi R, Soufflet C, Quadrio I, Yan ZX, Huyot V, Aubenque C, Destrez P, Roth K, Roberts C, Favero M, Clayette P. Inactivation of animal and human prions by hydrogen peroxide gas plasma sterilization. *Infection control and hospital epidemiology*: 2009;30:769-777
- (3) Doundoulakis JH. Surface analysis of titanium after sterilization: role in implant-tissue interface and bioadhesion. *J Prosthet Dent* 1987;58(4):471-8.
- (4) <http://www.harrickplasma.com/>

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