Laser micro-grooved, Arginine-Glycine-Apspartic acid (RGD) coated dental implants, a 5 years radiographic follow-up

Mohamed Ahmed Alkhodary BDS, MS, PhD
Lecturer of Removable Prosthodontics, Faculty of Dentistry, Alexandria University, Egypt
Assistant Professor of Prosthetic Dental Science, College of Dentistry, Al Qassim University, KSA.

Abstract:

Objectives: This work has utilized laser direct writing to produce 10 microns wide uniform grooves on the surface of custom made titanium (Ti-6Al-4V) dental implants, and the tri-peptide RGD coating to produce a micromechanical and a chemical union with the tissues around the implant crest module and minimize crestal bone loss. The aim of this study was to follow these implants radiographically after five years of service under a mandibular overdenture.

Methodology: Standardized digital periapical radiographs and the computer software "Image J" were used to evaluate the bone density profile and vertical bone loss along the mesial and distal sides of the implants used in this study.

Results: The results of this study demonstrated less vertical bone loss and higher bone density profiles next to the laser micro-grooved implants coated with the RGD than those only having the laser micro-grooves.

Conclusion: The RGD coating has improved the bone density profile and reduced the vertical bone loss around the studied dental implants. However, further studies are needed to compare the effects of the laser micro-grooves versus other uniform or non uniform surface features; also, the RGD coating should be compared to other biomimetic surface coating materials.

Key words: titanium, dental implants, laser micro-grooving, laser micromachining, direct laser writing, RGD.

Correspondence:

Mohamed A. Alkhodary BDS, MS, PhD
Head of the Faculty Development Unit,
Assistant Professor of Prosthetic Dental Science,
Board Member, Dental Research Center,
College of Dentistry, Al Qassim University, KSA.
Lecturer, Faculty of Dentistry, Alexandria University, EGYPT.
Visiting Graduate Fellow, MAE dept, Princeton University, USA.
Phone: 00966 538950814
Email: dr.mohamed.alkhodary@qudent.org

Introduction

Micro-machining and laser micro-texturing of Titanium-Aluminum-Vanadium (Ti-6Al-4V) have been used to produce relatively uniform micro-grooves on a wide range of materials. [(1-7)] The theory of micro-patterning finds some of its roots at Princeton University, where Soboyejo et al [(8-11)] have demonstrated that by controlling the size of laser machined grooves, cells can be induced to grow into the grooves and orient themselves accordingly. This phenomenon is known as contact guidance and has important implications. Contact guidance can be used to regulate where bone grows, and ultimately, what sort of bone develops. This spatial and material control means that isotropic and anisotropic properties can be controlled and that osseointegration can be promoted. Moreover, there is evidence that suggests that the groove geometry plays a role in determining what sort of tissue develops. For instance, 10 microns grooves are thought to induce bone stem cells to differentiate into cortical bone.

In biological terms, RGD is a cell adhesion receptor molecule that interacts with membrane bound talin and vinculin proteins to promote the cellular surface adhesion process. In other words, RGD's role is to accelerate the process of tissue binding to the implant surface. This has been empirically demonstrated, with up to a two-fold increase in cellular adhesion strength after 12 hours of cell culture time. This boost to cellular adhesion in the first critical period after surgery is vastly important in accelerating the healing process and strengthening the level of initial osseointegration.[(12-17)]

Long-term follow-up studies and consensus statements regarding implant survival and complications may provide more reliable insights about bone loss around the implants under over dentures. [(21-23)] Calvo-Guirado et al [(21)] measured the mean marginal bone loss the day after implant placement and after 1 year, and the values were 0.76±0.18mm. Schwartz-Arad et al [(22)] found that 70 percent of their patients with implant-supported overdentures lost less than 2mm bone in the first year. Misch et al [(23)] found that only 6mm of bone will typically be lost over a five-year period.

The Pisa [(24)] Consensus suggested that the clinical assessment for each implant should monitor marginal bone loss in increments of 1.0 mm. Also, computer-assisted image analysis and customized x-ray positioning devices may be superior methods of measuring bone loss, but were not required for the criteria established at that consensus.The Pisa [(24)] Consensus has also stated that the term implant success may be used to describe ideal clinical conditions. It should include a time period of at least 12 months for implants serving as prosthetic abutments. The term early implant success is suggested for a span of 1 to 3 years, intermediate implant success for 3 to 7 years, and long-term success for more than 7 years.

The Pisa [(24)] consensus has also provided a scale for Implant Quality ofHealth, based on clinical evaluation: success, survival, and failure. The success category describes optimum conditions, the survival category describes implants still in function but not with ideal conditions, and the failure of an implant represents an implant that should be or already has been removed. Group I represent success and is considered optimum health conditions. No pain is observed with palpation, percussion, or function. No clinical implant mobility is noted in any direction with loads less than 500g. Less than 2.0 mm of radiographically crestal bone loss is observed compared with the implant insertion surgery. The implant has no history of exudates. The prognosis of Group I implants is very good to excellent. Group II implants are categorized as "survival" and have satisfactory health. They are stable, but show a history of, or potential for, clinical problems. No pain or tenderness is observed on palpation, percussion, or function. No observable mobility exists with loads less than 500 g. Radiographic crestal bone loss is between 2.0 and 4.0 mm from the implant insertion. The prognosis is good to very good, depending on the stable condition of the crestal bone. And finally, Group III implants are categorized as "failure" and these may show mobility, exudates, vertical bone loss to more than one half the length of the implant, and might be sensitive to percussion.

The implants used in this study were designed, manufactured, laser micro-grooved and RGD coated at Princeton

University-USA in 2008 (Fig.1 and 2). And in 2009, the implants were used in a human clinical trial to assist overdentures within a Ph.D. thesis that was submitted to the Faculty of Dentistry at Alexandria University, Egypt in 2010. [25] Before conducting the human trials, the surface treatments were characterized [26] and tested in experimental animals. [27] the aim of the current work is to evaluate, radiographically, these custom made dental implant, with laser grooving and the combined RGD coating, after 5 years of service under mandibular overdentures in completely edentulous patients.

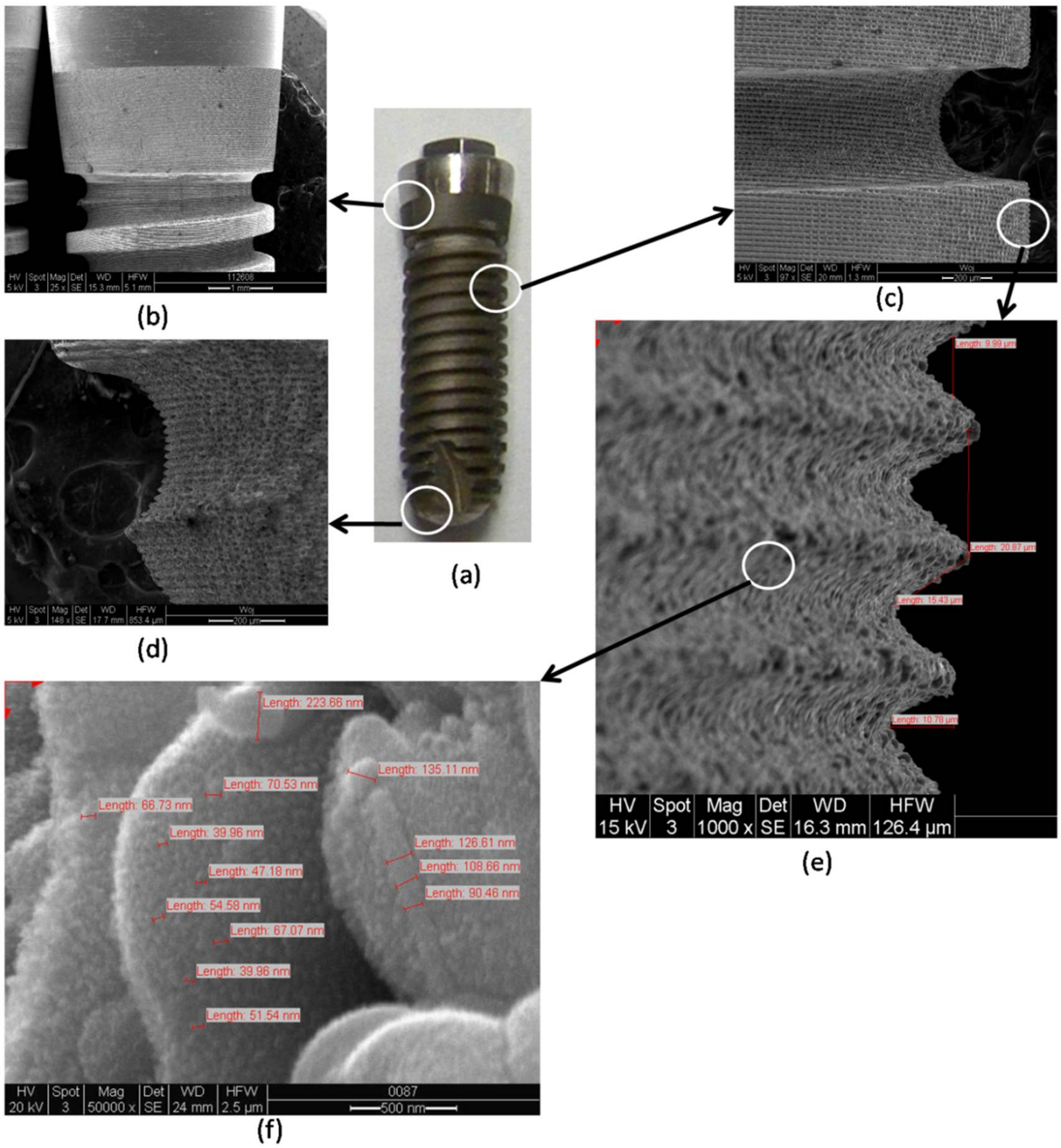


Figure (1): SEM of the laser microgrooved dental implant: (a) Macroscopic appearance of a laser micro-grooved implant, (b) SEM of the implant head, original magnification x25, (c)SEM of the threads, original magnification x97, (d) SEM of the foot, original magnification x148, (e)SEM of the laser micro-grooves, original magnification x1000, (f) SEM of the surface exhibiting roughness in the nano scale, original magnification x50,000.

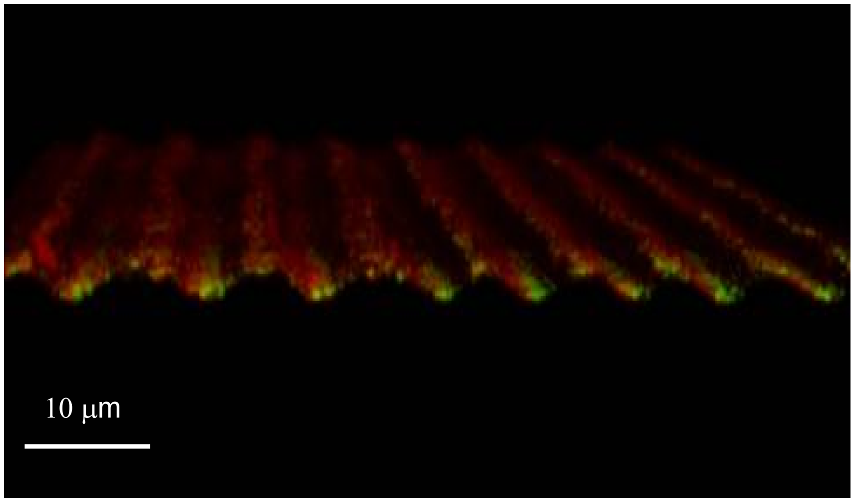


Figure (2): Fourier Transform Infrared (FT-IR) spectroscopy: Immunofloursecent Microscopy Image of Lasergrooved Surface Functionalized with the Alkyl Phosphonate (AP), the Linker Molecule to the RGD coating.

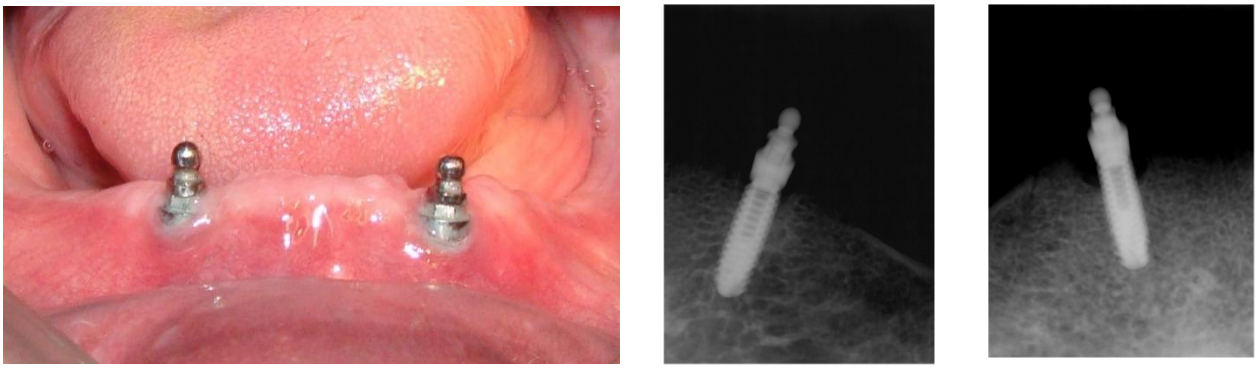
Materials and methods

Originally 6 completely edentulous male patients, ranging from 40 to 60 years old, volunteered to participate in this study, the procedures were explained to each one of them, and they all signed an informed consent. Each patient received two implants, and a mandibular complete overdenture; the right implant was laser micro-grooved and RGD coated, while the left implant was laser micro-grooved only. Both implants had a ball abutment that fitted within an open top metal housing and an elastic O-ring in the overlying overdenture.

However, only 5 patients were followed-up radiographically after 5 years. For each case, standardized digital periapical radiographs were taken using a film holding device for extension cone paralleling technique. (Fig. 3a-c)

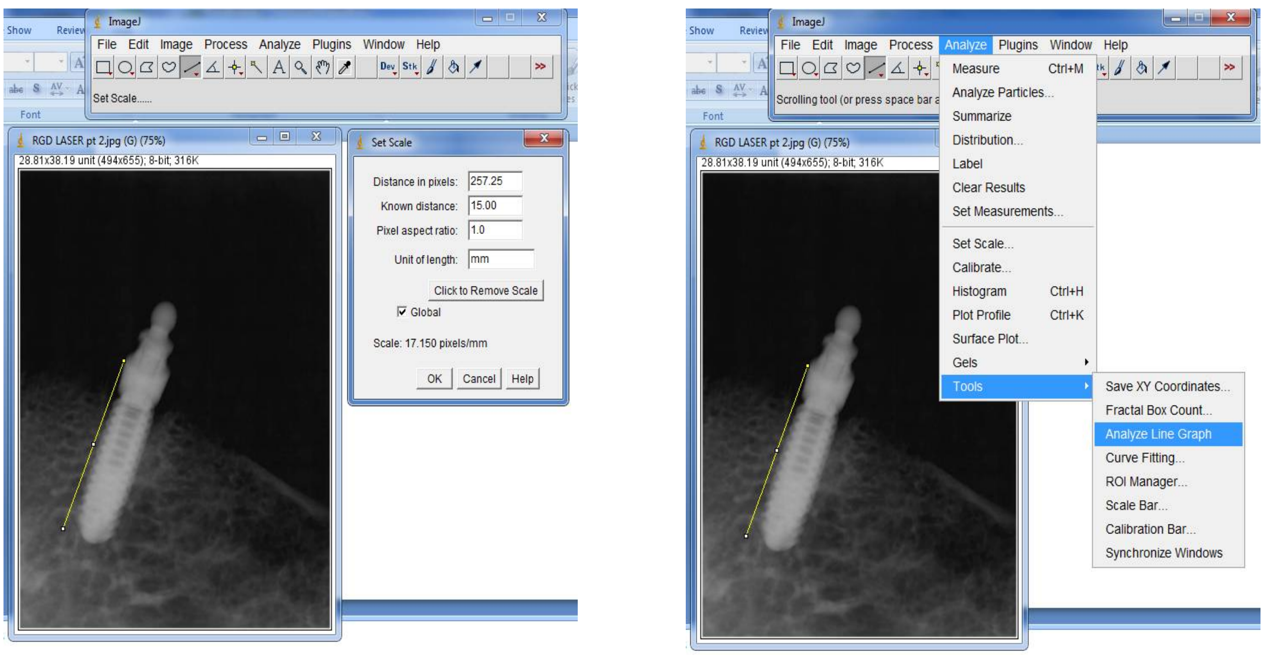
The computer software “Image J” (Image J 1.4 public domain software downloaded through the internet from the National Institute of Health (NIH, USA.)was used to determine the bone density profile along the mesial and distal sides of each implant, on a gray scale of 0 to 255.A line was drawn next to the implant from itsshoulder to the apex, the measurements were noted in millimeter both mesially and distally and, the mean for the 5 cases of each group was calculated.

The length of the implant (15 mm) was measured on the x-ray in order to determine the magnification factor in the radiograph. The measurements of the bone density were then adjusted according to the magnification (Fig. 4). From the analyze command, the analyze line graph was selected from the tools menu, then the selection was added and saved to be used in the follow up image for the same patient. The pixel aspect ratio selected was 1.0, and the measurement unit was a millimeter, and so, for the 15 mm length of the implant, there were 15 pixel regions, and themean Gray Value (average gray value within the selection) was the sum of the gray values of all the pixels in the selection divided by the number of pixels.



(a) (b) (c)

Figure (3): clinical and radiographic views of the implants used in this study: (a) A clinical photograph of the implants after 5 years. (b) Digital periapical photograph of the right implant. (c) Digital periapical photograph of the left implant.



(a) (b)

Figure (4): Bone density profile measurement: (a) A line is drawn along the side of the implant from its shoulder to its apex, the already known length of the implant (15 mm) was used as a guide to overcome any magnification effect, and a pixel aspect ratio of 1.0 was used, (b) The mean grey value for each of the 15 pixel regions for both the mesial and distal sides was calculated.

Results

The mean Gray Value representing the mean of bone density profile along both the mesial and distal sides of each implant was calculated, and then the mean for all the cases in each group was also calculated (Fig. 5). The results were tabulated and compared using the IBM SPSS software package version 20.0. [28]

For the right implants, the first 5 regions, representing the first 5 mm starting from the shoulder of the implants, did not show any bone. Then, the bone started to appear at region 6. Accordingly, the amount of vertical bone loss could be calculated by deducing the 1.5 mm polished collar, and it was 3.5± 0.45 mm.

For the left implants, the first 6 regions did not show any bone, and so, the amount of vertical bone loss could be calculated, by deducing the 1.5 mm polished collar, and it was4.5± 0.20 mm.

When comparing the right and left implants it was found that: in region 6, bone was detected only around the right implants, and then, in regions 7, 8 and 9, the mean gray values representing the mean of bone density profile were significantly higher around right side implants compared to the left side implants at $p \leq 0.05$. Finally, there was no statistically significant difference between the remaining regions.

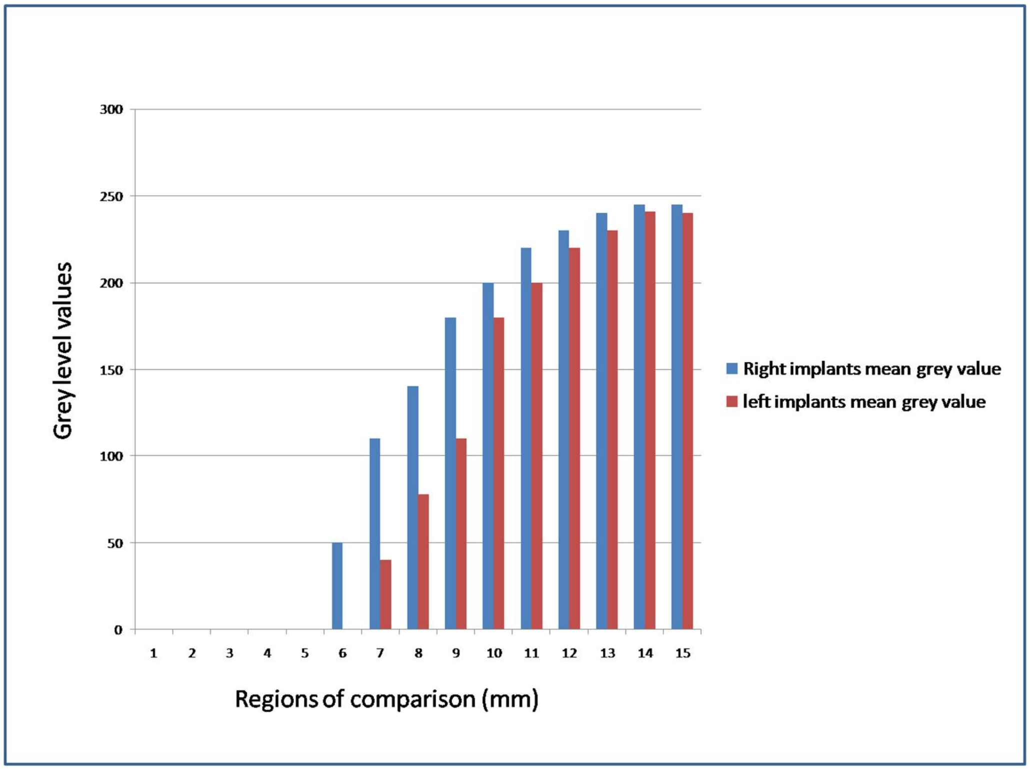


Figure (5): Grey scale values representing the mean bone density profile for both mesial and distal sides of of the implants in the two groups.

Discussion

This study was conducted in a trial to improve the adhesion of the bone to the implant surface both micromechanically and chemically, with consequent better stress distribution to the surrounding bone, and ultimately, a better peri-implant bone healing and remodeling that provides a long term support of the implant and the prosthesis it retains.

Several methods exist to render the titanium surface rough creating random uncontrolled surface roughness. [29-31] However, recently Souza et al [32] and Chhuchar et al [33] have shownthat laser micro grooving of titanium surfaces provided a controlled pattern that can be used to direct the cell orientation within predetermined grooves that correspond to their body size, with consequent better arrangement of the cells at the implant-bone interface and a better peri-implant bone formation.

Other strategies adopts the use of a surface coating material, pure protein extracts, drugs or chemical molecules to improve osseointegration, coating the implant with a receptor molecule known as RGD, which is based on the fact that bone sialoprotein (BSP-II) exhibits RGD sequence that binds to the vitronectin-type integrin receptor of osteoblasts, was found to promote attachment, adherence, differentiation and proliferation of osteoblasts. This comes in agreement with the findings of Rezania and Healy, [34] Kroese-Deutamn et al, [35] and Elmengaard et al [36, 37] who have reported significant increase in bone formation, or bone ingrowth in the RGD-Ti group compared with the Ti group. However, Dee et al [38] have questioned this finding claiming that RGD mediated adhesion does not account completely for osteoblast adhesion.

Five years after loading of the implants used in this study, the bone density profile values on the mesial and distal sides of the laser grooved/RGD coated implants were significantly higher than those of the laser grooved implants. These statistically significant findings proves the potential of the RGD coating in achieving better mineralization at the bone implant interface, and consequently higher values of the bone density profiles recorded. However, vertical bone loss was detected in both groups, being less by a 1± 0.25 mm around the laser grooved/RGD coated implants.According to the Pisa [24] consensus,both implants have achieved an intermediate implant success based on their survival for 3 to 7 years, and categorized as group II “survival and have satisfactory health” on the implant quality of health scale.

The vertical bone loss occurred can be attributed to a design parameter used, the non-platform switching, which is according toPaul et al[39] and Anandetal [40]can increase the crestal bone loss. A second reason for vertical bone loss would be theestablishment of a biological width, as dictated by Misch, [41] to be in the form of bonelossprocess progressing not only apically along the vertical axis, but also 1 to 1.5 mm horizontallyuntil the biological width has been created and stabilized. Finally, titanium stiffness, having a higher modulus of elasticity than the surrounding bone, results in a stress shielding phenomenon, [42, 43] that is the confinement of the applied stresses within the implant, and little dissipation to the surrounding bone, and this in turn, minimizes the mechanical stimulation that would otherwise encourage the maintenance of bone architecture and strength.

Conclusion

The results of this study demonstrated less vertical bone loss and higher bone density profiles next to the laser micro-grooved implants coated with the RGD than those only having the laser micro-grooves. Further studies need to be conducted to compare the results of this study and other studies using titanium dental implants with random surface roughness, to validate the hypothesis of contact guidance, and other surface coatings to compare the effect of RGD surface functionalization. Finally, researchers should be encouraged to seek new materials that possess similar biocompatibility to that of titanium, or even better, and a more matching modulus of elasticity to that of the bone to avoid the stress shielding process.

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Conflict of Interest

Author states that no conflicts of Interest exist.

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