

## COMPARISON OF THREE DIFFERENT DENTAL IMPLANT BIOLOGICAL COATING TECHNIQUES

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### ABSTRACT

**Introduction:** Titanium alloy (Ti-6Al-4V) surface coating with the amino acid RGD peptide was found to improve the adhesion of osteoblasts to the implant surface. This work aimed at comparing three different methods to immobilize the amino acid sequence RGD to the Ti-6Al-4V.

**Materials and methods:** This article tested three different methods to immobilize the amino acid sequence RGD to Ti-6Al-4V implants surface, the first method used alkane phosphonic acid and maleimide as coupling factor, the second method used a simple dip-in technique and dehydration, and the third method used a collagen coating to which the RGD was linked, then the resulting coatings were tested using the Fourier Transform Infrared (FT-IR) spectroscopy, and surface binding energy.

**Results:** The FT-IR and surface binding energy indicated the first method yielded better and stronger RGD coating.

**Conclusion:** The titanium alloy chemical coating process of the amino acid sequence RGD was found to yield better results as compared to the dip-in and RGD-collagen linking methods.

**KEY WORDS:** Dental implants, biomimetic coating, RGD coating.

### INTRODUCTION

Titanium alloy (Ti-6Al-4V) surface coating with the RGD peptide was found to improve the adhesion of osteoblasts to the implant surface,<sup>1-10</sup> which enhances the process of bone healing around dental implants, specifically the biomechanical properties of the developing bone/implant interface,

through enhancement of osteoprogenitor cells osteogenic potential, and promotion of existing osteoblast integrin-specific adhesion to the titanium implants surface.<sup>11-15</sup> However, the RGD coating methods were reported to be complex and technique sensitive.<sup>16-18</sup> The current work compared three of the most commonly used techniques regarding the quality of the resultant coating.

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## MATERIALS AND METHODS

Three techniques were used for RGD coating of Ti-6Al-4V dental implants, the study used 10 implants for each coating method.

The first technique immersed the samples in a solution of 3 mg alkane phosphonic acid (AP, Sigma Aldrich) in a 100 ml of Tetrahydrofuran (Sigma Aldrich) for 3-4 hrs. Then baked them at 120°C for 24 hrs. To achieve a coating of AP as seen in Figure 1. To add the Maleimide, the samples were then immersed in a second solution of 10 mg Maleimide (Sigma Aldrich) in 500 ml anhydrous Acetonitrile (Sigma Aldrich), and finally, the samples were immersed in a third solution of 0.5 mg of Arg-Gly-Asp- Cys (RGDC) dissolved in 500 ml distilled water under stirring condition for another 24 hrs.

The second method was a simple dip-coating process, where the implants were placed for 15 min in an RGD solution, and then dried in a dry heat oven for 5 hrs. at 30 °C. (Fig.2)

The third method coated the implants by a layer of collagen, then used a hetero-bifunctional linker, sulfo-SMPB (sulfo-succinyl-imidyl 4-(p-maleimidophenyl butyrate)) to connect thiol anchors that connected the RGD to the free amino groups of the collagen coating. (Fig.3)

The characterization of the surface coating techniques was done using the Fourier Transform Infrared (FT-IR) spectroscopy to detect the infrared emission the functional RGD groups attached to the surface of the 10 implants used in each of the three groups, and by the X-ray photoelectron spectroscopy (XPS, K-Alpha ESKA system; Thermo, USA) to detect the binding energy of the RGD coating to the surface in each of the three methods used. The 10 readings obtained from the FTIR and XPS for each coating methods were tabulated and the analysis of variance (ANOVA) was used to test the results of the three methods with p significance level  $\leq 0.05$ .



Fig. (1) The first coating method designed to suspend the implants in sequence of 3 solutions of alkane phosphonic acid, Maleimide, and finally Arg-Gly-Asp-Cys (RGDC).



Fig. (2). Implants in the second method dried in dry heat oven for 5 hrs. at 30 °C.



Fig. (3). The third method coated the implants by a layer of collagen, then immersion in RGD to solution.

## RESULTS

The FT-IR transmittance peak for the first method was found to occur at  $\sim 3350$ , and for the second method at  $\sim 2700$ , and for the third method at  $\sim 2750$  as seen in the Figure 4 where the three methods transmittances' curves were superimposed. The statistical analysis indicated that the first method had statistically significant higher peak than the other two methods, and that there was no statistically significant difference between the second and third methods.

Figure 5 shows the XPS spectra of first method maleimide linked RGD peptide as line 1, second method titanium oxide RGD coated as line 2, and third method collagen linked-RGD as line 3, where there also was statistically significant difference between the first method and the other two methods, which also had no significant difference between them.

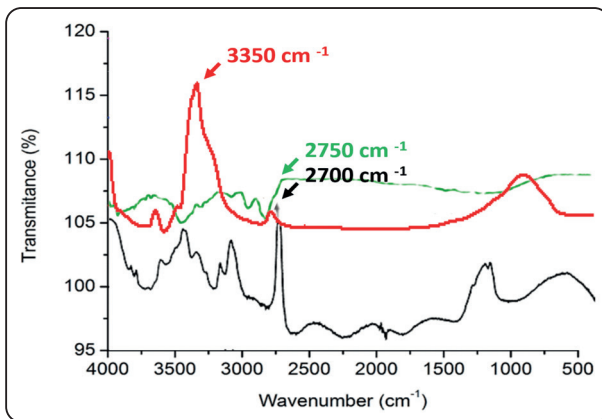


Fig. (4). Fourier Transform Infrared (FT-IR) spectroscopy peak for the first method (red colored curve), and for the second method (black colored curve), and for the third method (green colored curve).

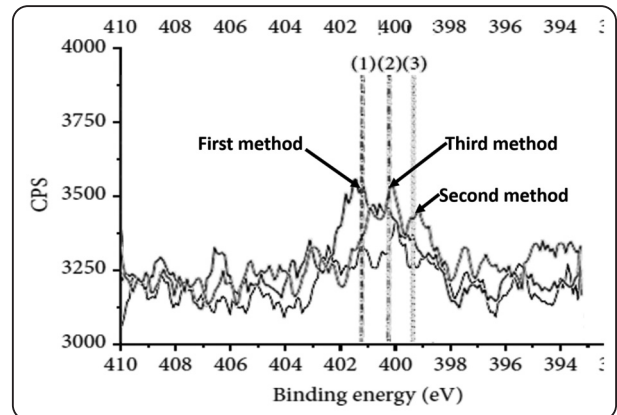


Fig. (5) X-ray photoelectron spectroscopy binding energy curves of the three coating methods.

## DISCUSSION

In the first coating method, the samples were coated with AP, then maleimide, that reacted with the cysteine of the R-G-D-C to yield a strong covalently bound organic coating, that was resistant to removal by sonication, solvent washing, or by mechanical peeling with tape as proven in the work of several studies.<sup>3-9</sup> On the other hand, and in contrast to the findings of Syam et al,<sup>10</sup> and Schliephake et al,<sup>18</sup> the surface coatings resulting from the second and third methods in this study did not have the same FT-IR peak intensities or surface binding energy, in addition to the possibility of their removal by friction during implant insertion in the osteotomies, a fact that further favors the use of the first method in spite of its sensitivity and complexity. However, taking the complexity of the first coating method into consideration, other methods for RGD surface immobilization tried in other studies had a more complex nature, for example, Georgieva et al,<sup>11</sup> used a gelatin solution and ultrasound to deposit the RGD sequence, which represented a more complex and sensitive method than those used in this study, and Chen et al,<sup>12</sup> who incorporated the RGD in a poly-amino acid coating to improve the performance of orthopedic implants, resulting a surface porous structure which had

questionable mechanical properties as compared to the chemically bound coating achieved by the first method used in this study. Additional innovation in RGD coating method included the work of Ma et al,<sup>13</sup> who loaded extracellular vesicles with RGD, and then attached these vesicles to the titanium surface with a specific bonding peptide, a method that represented an even more challenging, and an indirect method to achieve such biological coating.

## CONCLUSION

The limitations of the current work might include the followings:

1. The study could have tested several other coating methods.
2. The study could have incorporated more surface characterization methods.
3. The study could have included more samples, however, the 3 methods tested in the current work represented 3 of the most commonly used method, and the results of this study confirm previous findings of a clinical trials, and experimental animal studies that reported success of the RGD coating to titanium implants.<sup>16,17</sup>

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