HOS cell adhesion on Ti6Al4V ELI texturized by CO2 laser

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Abstract. In this work, the response of HOS cells on Ti₆Al₄V ELI textured surfaces by a CO₂ laser was evaluated. The test surfaces were; smooth Ti₆Al₄V, used as the control, and four textured surfaces with linear geometry. These four surfaces had different separation distances between textured lines, D₁ (1000 microns), D₂ (750 microns), D₃ (500 microns) and D₄ (250 microns). Toxicity of textured surfaces was assessed by MTT and the cellular adhesion test was performed using HOS ATCC CRL 1543 line cells. This test was done after 5 days of culture in a RPMI 1640 medium supplemented with 10% fetal bovine serum and 1% antibiotics. The results showed that the linear textures present 23% toxicity after 30 days of incubation, nevertheless, the adhesion tests results are inconclusive in such conditions and therefore the effect of the line separation on the cell adhesion cannot be determined.

1. Introduction
Due to their excellent properties, titanium and its alloys have been established as the most widely used materials for manufacturing medical devices. This type of material has a clearly defined market in bone fixation and replacement applications. Due to the bioinert behaviour of the titanium surface, the need to implement different surface modification techniques have been generated in order to improve the osseointegration process of this material [1-4]. Thermal and chemical oxidation, cathodic electrodeposition, plasma electrolytic oxidation and laser modification are some of the most widely used surface modification methods.

Recently some authors have suggested that the osseointegration process of the implant material can be favoured by increasing surface roughness or texture [5-9]. An easy-to-apply method for the surface texturizing of these materials is laser texturizing, since it creates a great variety of surface patterns accurately, quickly and with excellent repeatability [5,10]. Therefore, the purpose this study is to analyse the effect of laser texturizing distance separation patterns using a CO₂ laser in the adhesion of HOS line osteoblasts on Ti₆Al₄V ELI surfaces.

2. Methodology
Ti₆Al₄V discs with 14mm diameter and 3mm thickness were used. The disks were chipped with silicon carbide paper (1200 grit) and cleaned ultrasonically with ethanol for 10min. Afterwards, disks were modified by laser texturing using a laserpro X380-RX equipped with a CO₂ laser. The patterns generated on the discs of the titanium alloy had 100 microns wide lines, with 4 different distances between lines (1000μm, 750μm, 500μm, and 250μm). The power used for the laser texturing was 40W. After the texture process, the samples were viewed in an Olympus BX41 optical microscope, to observe the morphology of the surface, in addition to the surface reconstruction in 3D with the aid of the Zen lite program.
The biologic response of the specimens was determined by the quantitative adhesion of human osteosarcoma cells (HOS, ATCC CRL-1543), following the methodology previously described in [9,10]. The HOS cells were kept in RPMI supplemented with 10% fetal bovine serum and 1% penicillin / streptomycin in an atmosphere of 5% CO2, 95% humidity and a temperature of 37°C. Cells were sub-cultured once they had a 90% cell confluence (3 days culture) by trypsin-EDTA peeling. Textured surfaces D1, D2, D3 and D4, previously sterilized in autoclave at 121°C, for 30 minutes at 15psi, were placed inside 24 well plates and 1.5×10^4 cells/mL were grown on their surface. After 5 days of incubation the adhered cells were detached with tryps in-EDTA and centrifuged at 1500rpm for 5 minutes. The quantification was carried out re-suspending the pellet with yellow eosin and counting the viable cells by optic microscopy with the Neubauer chamber. Surfaces without texturizing Ti₆Al₄V were used as control.

3. Results

As can be seen in the micrographs of Figure 1, notice that the textured lines are homogeneous along the surface of the Ti₆Al₄V alloy. The clear areas present in the micrograph are the areas of the alloy that were not exposed to the texturing process; there are some smooth lines that are product of the surface preparation process. The dark areas are due to the laser-textured process. This process induces a black coloration of the material due to the implementation of the Cermark coating, which must be applied to the surface following current standard protocols to generate textures with the laserpro X380-RX equipment. Additionally, as the separation distance between lines decreases, an increase in the apparent width of the lines can be seen because the Cermark coating generates black marks outside the lines generated.

![Figure 1](Image)

**Figure 1.** Optic micrograph of the Ti₆Al₄V texturized surfaces (a) 1000µm, (b) 750µm, (c) 500µm and (d) 250µm at 5X.

With the implementation of Zen lite software, 3D reconstruction of the textured surfaces was carried out. As can be seen in Figure 2 the textured process generates an increase in surface roughness, which could favour the adhesion of the bone cells.
The toxicity tests show that line textured surfaces generate a toxicity of 23%. This result is attributed to the fact that the texturing process is causing the inclusion of new elements to the surface as has been previously reported in [10]. This material incorporated into the surface of the Ti6Al4V alloy can affect the toxicity of the alloy. On the other hand, when performing the cell adhesion test it was found that the textured titanium alloy surfaces have high affinity with HOS cells adherence. This increase in adhesion could be explained by an increase in roughness due to the texture of the material. In most cases it was possible to quantify the number of adhered cells by this method. However, surface D3 showed the best adhesion result with a value of $6 \times 10^3$ cells. Figure 3 shows individual results of the cells adhered in each of the replicates of the surfaces of the modified material. On the other hand, Figure 4 shows the percentage of viable cells when exposed to the vital yellow dye eosin. The results of each of the samples are shown independently.

![Figure 2. 3D reconstruction of Ti6Al4V texturized surfaces (a) 1000µm, (b) 750µm, (c) 500µm and (d) 250µm.](image)

![Figure 3. Cell adhesion on Ti6Al4V texturized surfaces, D1=1000µm, D2=750µm, D3=500µm and D4=250µm.](image)

![Figure 4. Cell viability on Ti6Al4V texturized surfaces, D1=1000µm, D2=750µm, D3=500µm and D4=250µm.](image)
The results showed that the textured Ti₆Al₄V alloy samples have good affinity with HOS cells. Besides, due to the high toxicity percentage found by the MTT assay and the low viability reported in the adhesion tests, it is not possible to conclude the effect the separation distance of the textured lines by laser on the titanium alloy. When comparing the results of this study with projects previously developed by Sandoval et al. [9-10] where circular morphology textures were used, it is possible to conclude that due to the size of the lines and to the inclusion of a large number of elements resulting from the Cermark surface coating an unfavourable response occurs by the lined textured surfaces to the cell adhesion process.

4. Conclusions
Patterns obtained by laser texturizing using a CO₂ laser do not produce a cytotoxic response, nevertheless, the results of the adhesion tests are inconclusive and do not allow the effect of the line separation on the cell adhesion to be determined. Therefore, it is recommended to continue the study of the texture geometry effect on the cellular response.

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References
[1] Pfleging W, Kumari R, Besser H, Scharnweber T and Majumdar J D 2015 Appl. Surf. Sci. 355 104
[2] Hu T, Hu L and Ding Q 2012 Surf. Coat. Technol. 206 5060
[3] Qin Y, Xiong D and Li J 2015 Surf. Coat. Technol. 269 266
[4] Fasasi A Y, Mwenifumbo S, Rahbar N, Chen J, Beye A C, Arnold C B and Soboyejo W O 2009 Mater. Sci. Eng. C 29 5
[5] Kumari R, Scharnweber T, Pfleging W, Besser H, and Majumdar J D 2015 Appl. Surf. Sci. 357 750
[6] Chen J, Ulerich J P, Abelev E, Fasasi A, Arnold C B and Soboyejo W O 2009 Mater. Sci. Eng. C 29 1442
[7] Györgyey A, Ungvári K, Kecskeméti G, Kopniczky J, Hopp B, Oszko A, Pelsöcsi I, Rakonczay Z, Nagy K and Turzo K 2013 Mater. Sci. Eng. C 33 4251
[8] Liang C, Wang H, Yang J, Cai Y, Hu X, Yang Y, Li B, Li H, Li H, Li C and Yang X 2013 Appl. Mater. Interfaces 51 8179
[9] Sandoval Amador A, Montañez Supelano N D, Vera Arias A M, Escobar Rivero P and Peña Ballesteros D Y 2017 J. Phys.: Conf. Ser. 786 012010
[10] Sandoval Amador A, Carreño García H, Escobar Rivero P and Peña Ballesteros D Y 2016 J. Phys.: Conf. Ser. 687 012012