HOS cell adhesion on Ti₆Al₄V surfaces texturized by laser engraving

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Abstract. The cell adhesion of the implant is determined by the chemical composition, topography, wettability, surface energy and biocompatibility of the biomaterial. In this work the interaction between human osteosarcoma HOS cells and textured Ti₆Al₄V surfaces were evaluated. Ti₆Al₄V surfaces were textured using a CO₂ laser in order to obtain circular spots on the surfaces. Test surfaces were uncoated (C1) used as a control surface, and surfaces with points obtained by laser engraving, with 1mm spacing (C2) and 0.5mm (C3). The HOS cells were cultured in RPMI-1640 medium with 10% fetal bovine serum and 1% antibiotics. No cells toxicity after one month incubation time occurred. The increased cell adhesion and cell spreading was observed after 1, 3 and 5 days without significant differences between the sample surfaces (C2 and C3) and control (uncoated) at the end of the experiment.

1. Introduction
Some papers have shown that the adhesion, spreading and proliferation process depend on the physicochemical surface properties. Composition, electric charge, material conductivity, roughness, and micro or nanopatterning play an important role in the cellular response of the material when it is implanted [1-3]. For example, the chemical composition of the surface determines the surface energy, polarity, wettability, Zeta potential and consequently the characteristics of the cell–material interaction [4-6]. Therefore, a suitable surface treatment can promote osteosynthesis ability of the implant material, so producing cells to adhere and proliferate on the major surface and likewise lead to an increase in mineralization of the surface, which forms the basis for the formation of new bone [7].

In this work the effect of the geometry of a pattern obtained by laser engraving on the adhesion of human osteosarcoma cells on surfaces of Ti₆Al₄V was evaluated.

2. Experimental

2.1. Samples
A mill annealed Ti₆Al₄V cylindrical disc with 14mm diameter and 3mm thick was used to study the patterning by laser engraving effect on cells/surface interactions. Three different types of surfaces were used in this study. They include: smooth surface (C1) and laser engraving with two different circular patterns (C2 and C3). The smooth surface was obtained by grinding with silicon carbide paper in successive grades from 220 to 1200 grit and subsequently polished with slurry containing 3µm and 0.5µm Al₂O₃ powder. The patterning surfaces were obtained by laser engraving using a laserpro X380-RX equipment; this has a sealed CO₂ laser with maximum power of 100W. The circular patterns...
consist of points with 100µm diameter, 1mm vertical spacing between points, 1mm horizontal distance for pattern 1 and 0.5mm for pattern 2. All samples were sterilized by UV radiation for 30 minutes on each side and then autoclaved at 120°C for 20 minutes.

2.2. Biological test
The human osteosarcoma cell line (HOS, ATTC, CRL-1543) was cultured in 25cm² Falcon Culture plates and maintained in an incubator at a temperature of 37°C regulated with 5% CO₂, 95% air, and saturated humidity. A Roswell Park Memorial Institute medium (RPMI-1640) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin was used as the cell cultured medium.

For cell toxicity assay, each sample (C2 and C3) and controls (sample without coating, C1) were placed in 1.5ml of RPMI-1640 culture medium and incubated at 37°C. After a month, the culture medium was recollected and HOS cells were incubated with serial dilutions of each supernatant or medium alone for 72 hours at 37°C. Cell toxicity was evaluated using a colorimetric MTT assay as described by J. E. Gomez et. al [8]. The percentage of cytotoxicity was calculated with the equation: cytotoxicity (%)=(OD control group-OD treatment)/OD control group x100.

For adhesion assay, 5.0x10⁴ cells/ml in cultured medium were placed on each material and incubated at 37°C. After 24, 72 and 120 hours the cells were detached using 0.5ml Trypsin-EDTA for 5 minutes and counted microscopically in a Neubauer chamber. In addition a fluorescence staining were made directly in each sample using Hoechst 33342 fluorescent stain and epifluorescence microscopy (Nikon Eclipse E4000), B-2A filter (Ex=450-490, DM=500, Ba=515). Images were obtained using a camera Nikon Coolpix 5000. UV2A. Each time experiments were performed in duplicate and the results were calculated as number of cells per surface area.

3. Results and discussion
The surface morphology of Ti₆Al₄V smooth C1 and patterned C2 and C3 shown in the Figure 1. C1 sample has a smooth and homogeneous surface like a mirror finish surface. Also on surface of C2 and C3 it can be observed that the patterns obtained, have a quasi-circularity shape attributed to sweep the laser beam on the surface to generate this kind of geometry. The diameter of the spots is about 150µm, the separation action on center between the spots on the vertical axes is about 1370µm and is the horizontal separation of 1475µm for C2 and 730µm for C2 and C3.

![Figure 1](image) Ti₆Al₄V surface morphology (a) smooth C1, (b) Pattern 1 C2, and (c) Pattern 2 C3.

As shown in the Figure 2, the spots have a dendritic structure from the center to the edge. This maybe is due to rapid heating cooling cycle during the laser engraving process. Furthermore, the high temperature can be caused by absorption of a large number of photons in the small area of the spot, producing then combustion of the material and moving this to the vicinity of the spots, this has been previously reported by M. E. Khososhahi et. al [9].

The contact angle results showed that the surfaces of C1, C2 and C3 samples have a hydrophilic behaviour, since the angles are less than 90°. As shown in the Figure 3, the laser engraving generated a change in the wettability of the surface, making the C3 surface slightly more hydrophobic. There were
no statistically significant differences between the contact angle of C1 and C2 but respect C3 surface the difference is around 43% calculated.

**Figure 2.** Pattern surface morphology, (a) Pattern 1 C2 and (b) Pattern 2 C3.

**Figure 3.** Contact angle.

Surface of Ti₆Al₄V with and without laser engraving were not toxic to the HOS cells any evaluated concentration, which is consistent with that reported by P. Sevilla et. all in [10], as shown in Figure 4, the percentage of cytotoxicity are below 15%.

**Figure 4.** MMT Assay.

The results of cell adhesion are shown in the Figure 5. At third day, C3 surface have a low adherence respect at the other surfaces. The number of attached cells increased exponentially on the
fifth day but there were no significant statistically differences in the cell adhesion on the studied surfaces. Although the number of adherent cells on the surfaces at the first day was very low, these few cells adhered with a good cell affinity for the three surfaces. Cells showed oval shape at first day and become each day more elongated which indicates the spread of the cells on the surfaces and it can be observed in Figure 6, these results are similar to those reported in [9].

Figure 6. Fluorescence micrographs of HOS cells attached on different studied surfaces.

4. Conclusions
The patterns obtained in C3 achieved modify the wettabilty of the surface alloy by 43% which is associated to the number of spots on the surface. Thus the patterns obtained by laser engraving, not produce a cytotoxic response; therefore the biocompatible nature of alloy is maintained.

The cell proliferation in three surfaces exhibit good cell adhesion with random orientations, however, no significant differences in cell adhesion of HOS cells by comparing the samples with and without pattern were observed, which concludes that requires extending the study variables to evaluate this method of surface engineering for osteosynthesis process.

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