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Relevant aspects in the surface properties in titanium dental implants for the cellular viability

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ABSTRACT

Roughness and topographical features are the most relevant of the surface properties for a dental implant for its osseointegration. For that reason, we studied the four surfaces more used in titanium dental implants: machined, sandblasted, acid etching and sandblasted plus acid etching. The roughness and wettability (contact angle and surface free energy) was studied by means 3D-interferometric microscope and sessile drop method. Normal human gingival fibroblasts (HGF) were obtained from small oral mucosa biopsies and were used for cell cultures. To analyze cell integrity, we first quantified the total amount of DNA and LDH released from dead cells to the culture medium. Then, LIVE/DEAD assay was used as a combined method assessing cell integrity and metabolism. All experiments were carried out on each cell type cultured on each Ti material for 24 h, 48 h and 72 h. To evaluate the *in vivo* cell adhesion capability of each Ti surface, the four types of discs were grafted subcutaneously in 5 Wistar rats. Sandblasted surfaces were significantly rougher than acid etching and machined. Wettability and surface free energy decrease when the roughness increases in sand blasted samples. This fact favors the protein adsorption. The DNA released by cells cultured on the four Ti surfaces did not differ from that of positive control cells ($p > 0.05$). The number of cells per area was significantly lower ($p < 0.05$) in the sand-blasted surface than in the machined and surface for both cell types (7 ± 2 cells for HGF and 10 ± 5 cells for SAOS-2). The surface of the machined-type discs grafted *in vivo* had a very small area occupied by cells and/or connective tissue (3.5%), whereas 36.6% of the sandblasted plus acid etching surface, 75.9% of sandblasted discs and 59.6% of acid etching discs was covered with cells and connective tissue. Cells cultured on rougher surfaces tended to exhibit attributes of more differentiated osteoblasts than cells cultured on smoother surfaces. These surface properties justify that the sandblasted implants is able to significantly increase bone contact and bone growth with very good osseointegration results *in vivo*.

1. Introduction

Over the last four decades dental implants have changed the face of dentistry. Dental implants are used as artificial replacements for the teeth roots and most of them consist of titanium (Ti). Dental implants are used to restore function and aesthetics in fully or partially edentulous patients [1–3]. Ti is considered to be an excellent material due to its physical properties such as stability, resistance and elasticity [4]. In addition, Ti implants are very stable *in vivo* and biocompatible, which can improve osseointegration [5]. However, cell adhesion to this type of material is not always strong, and new formulations and surface modifications should be developed to increase cell attachment to Ti implants [6]. In fact, it has been previously demonstrated that surface characteristics are one of key factors that determine the long-term success of dental implants [7]. In this regard; novel Ti-based biomaterials for use in dentistry include some modifications of the implant surface to increase biocompatibility and cell adhesion. In general, human bone and stromal cells have very low attachment capability to smooth metal surfaces, and the use of these materials could lead to infections, inflammation and low cell viability [8–10]. For this reason, modifications affecting roughness, topography and chemistry of the implant surface have been proposed to increase cell viability and biocompatibility [11]. The most common surface modifications used in dentistry are based on mechanical abrasion, sandblasting and acid etching [12]. Although some previous studies tried to evaluate the effects of these surface modifications on biocompatibility and functionality of dental implants by determining adhesion, migration and cell proliferation [13–14], the most appropriate modification for use on Ti dental

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implants has not been elucidated to the date. In addition, most of these studies are carried out on one single cell type such as fibroblasts, osteoblasts and epithelial cells [15–16], and most studies are restricted to the use of a limited number of methods and techniques to evaluate cell viability and cell function.

In this work, we have carried out an *ex vivo* analysis of cell behavior and function on four surface modifications of Ti implants using two different human cell types - stromal fibroblasts and a bone cell line - by using a combined array of methods and techniques to determine which Ti material modification could be more appropriate for future *in vivo* use.

2. Materials and methods

2.1. Titanium materials

The material studied was a commercial titanium alloy (Ti-6Al-4V ELI extra low interstitial medical grade). In this research, we used four different types of Ti materials subjected to surface modifications. Each material consisted in a disc of 12 mm diameter and 4 mm height whose surfaces had been treated by (A) mechanical abrasion, (B) sandblasting plus acid etching, (C) sandblasting or (D) acid etching.

All Ti6Al4V discs were sterilized by gamma irradiation and each cell type was cultured on the top surface of each disc type at a concentration of 88,500 cells/cm^2 (100,000 cells per disc) using culture medium. Each cell type was maintained in each Ti material for 24 h, 48 h and 72 h at 37 °C with 5% CO_2 in a cell incubator.

2.2. Roughness

Roughness was determined by means of a white light interferometer microscopy (Wyko NT1100, Veeco). The surface studied was 459.9 × 604.4 μm^2 for all samples. Data analysis was performed with Wyko Vision 232TM software (Veeco, USA). A Gaussian filter was used to separate waviness and form from the roughness of the surface. The following cut-off values were applied: $\lambda_c = 0.8$ mm, for micro-rough surfaces and $\lambda_c = 0.25$ mm for control surfaces. The measurements were realised in four different surfaces for each type of treatment to characterize the amplitude and spacing roughness parameters S_a and P_c, respectively. S_a and P_c were calculated by averaging the values of each profile that were evenly distributed along the surface analysed. S_a (the average roughness) is the arithmetic average of the absolute values of the distance of all points of the profile to the mean line. P_c is the number of peaks in the profile per length of analysis. Finally, the index area is the calculated [(real area) / (nominal area)] ratio. The hybrid parameter was calculated from the total real surface instead of from the profiles, as the index area parameter should be used for the Wenzel correction introduced below.

Scanning electron microscope (Jeol 6400, Japan) was used to qualitatively to analyse the surface topography of the implants before being implanted.

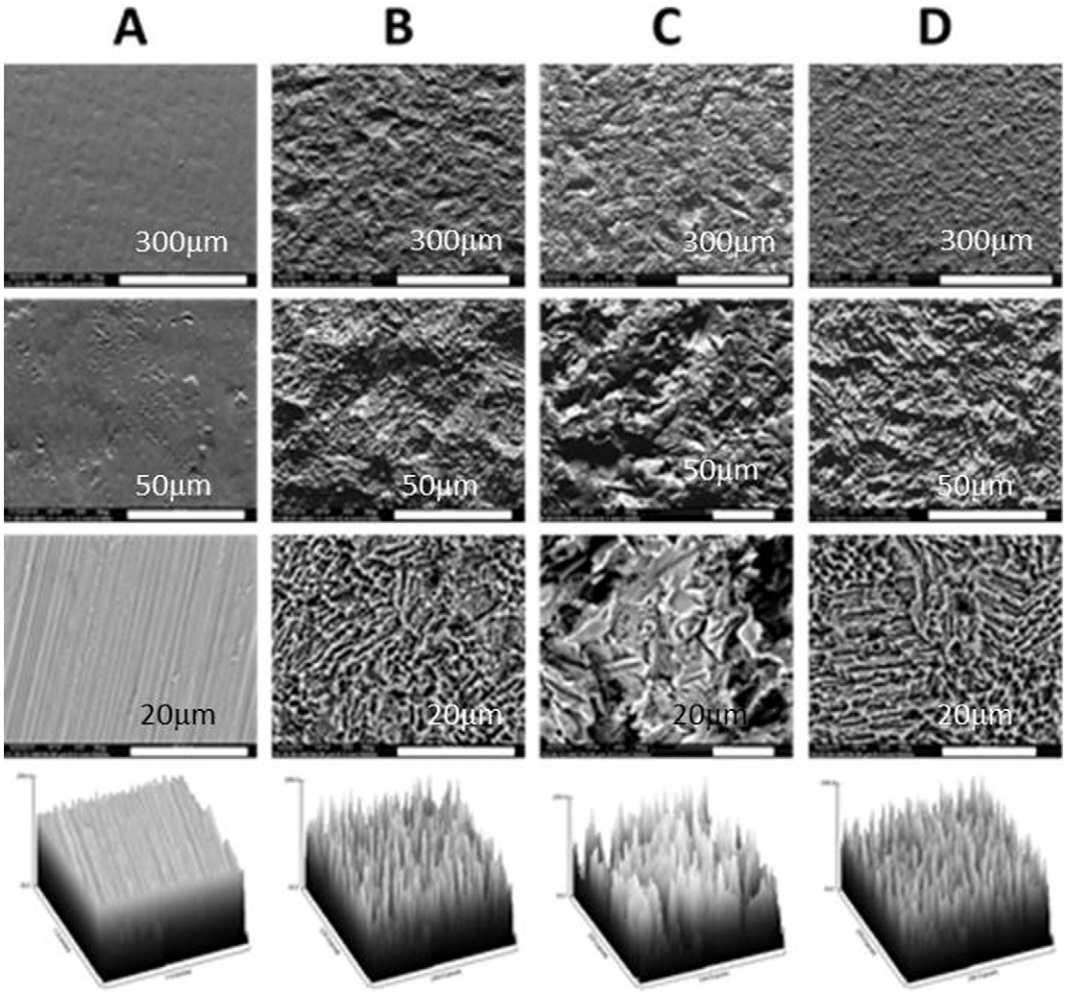

Fig. 1. Titanium surface analysis by scanning electron microscopy (SEM). In the first three rows, the four types of Ti discs (A (mechanical abrasion), B (sandblasting plus acid etching), C (sandblasting) and D (acid etching)) were analyzed at different augmentations. The lowest panel represents the three-dimensional representation of the analysis of surface plot for each material.