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Osteo Growth Induction titanium surface treatment reduces ROS production of mesenchymal stem cells increasing their osteogenic commitment

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ABSTRACT

Surface characteristics play a special role for the biological performance of implants and several strategies are available to this end. The OGI (Osteo Growth Induction) titanium surface is a surface, obtained by applying a strong acid onto the blasted surface. The aim of this in-vitro study is to evaluate in vitro the osteoproperties of OGI surfaces on Mesenchymal Stem cells derived from dental pulp. Our results confirm that this treatment exert a positive effect on mitochondrial homeostasis, as shown by a decrease in ROS production related to environmental stress on the mitochondria. Morphological and molecular biology analyses confirmed more over that the DPSC cultured on the OGI surfaces appeared more spread in comparison to those grown on control titanium surface and real time PCR and biochemical data clearly demonstrated the increase of osteoconductive properties of the OGI treatment. In conclusion, our results suggest that mesenchymal stem cells sensitively respond to surface properties related to OGI treatment enhancing their osteogenic activities.

1. Introduction

Implant dentistry has undergone a slow but steady growth during the last 40 years. When teeth are missing for caries, periodontal disease or agenesis, dental implants, such as dentures, are used either to replace the missing elements or to support complex prostheses [1].

The first generation of successfully used clinical titanium implants, which were machined with a smooth surface texture; now approach 40 years in clinical use. The second generation of clinically used implants underwent chemical and topographical modifications, usually resulting in a moderately increased surface topography. Many of these oral implant systems now approach 25 years of clinical use [2].

Surface characteristics play a special role for the biological performance of implants. Whereas mechanical properties such as Young's modulus and fatigue properties are mainly determined by the bulk of the material, chemical and biological interactions between the material and the host tissue are closely associated with the material surface properties [1]. Characteristics such as surface composition, surface topography, surface roughness, and surface energy affect the mechanical stability of the implant/tissue interface [3,4,5]. It was found that cell attachment and proliferation were surface roughness sensitive, and increased as the roughness of Ti-6Al-4V increased [6]. Many works have been carried out on surface treated commercial titanium implants to enhance the osteointegration function [7]. Experimental evidence from in vitro and in vivo studies strongly suggests that some types of surface modifications promote a more rapid bone formation than do machined surfaces. This could depend on an altered surface chemistry and or an increased texture on the micrometer scale [1,8,9]. Methods for altering surface texture can be classified as either techniques that add particles on the biomaterial, creating a surface with bumps (additive methods), and techniques that remove material from the surface, creating pits or pores (subtractive methods) [10].

The additive methods employed the treatment in which other materials are added to the surface, either superficial or integrated, categorized into coating and impregnation, respectively. Meanwhile, the subtractive techniques are the procedure to either remove the layer of core material or plastically deform the superficial surface and thus roughen the surface of core material. The common subtractive techniques are large-grit sands or ceramic particle blasts, acid etch, and anodization [1,11].

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The OGI (Osteo Growth Induction) titanium surface is a surface, obtained [13] by applying a strong acid onto the blasted surface. This treatment combines blasting with large-grit sand particles and acid etching sequentially to obtain macro roughness and micro pits to increase the surface roughness as well as osteointegration [7,14,15].

The aim of this in-vitro study is to evaluate in vitro the osteoproperties of OGI surfaces on Mesenchymal Stem cells derived from dental pulp; namely: Dental Pulp Stem Cell (DPSC) with a great attention to evaluate even long term cultures could affect the efficiency of the commitment of the cells. We'll here focus our effort also in evaluation on the anti-aging properties of the surfaces eventually exerted through variation on ROS production.

2. Material and methods

2.1. Biomaterial

This study cell activity on 2 implant surfaces was evaluated. We utilized implants with grit-blasted surfaces (control) and OGI surfaces (CLC implant, Italy). OGI samples are obtained by surface treatment by large grit sandblasting followed by double acid etching and relevant cleaning cycles.

The materials involved sandblasting medium is corundum and the double acid etching involves a mixture of mineral acids. In this process, the acids used in the double acid etching steps that follow sandblasting remove sandblasting particles

All experimental surfaces were cleaned and sterilized by γ-rays.

2.2. Analysis of the surface chemistry by XPS (X-ray photoelectron spectroscopy)

XPS analysis was performed using a Perkin Elmer PHI 5400 ESCA spectrometer. This is equipped with an X-ray source with an Mg anode, maintained at 20 kV with a nominal power rating of 200 W. The depth analyzed is approx. 5 nm. The pressure inside the analysis chamber has been maintained at approx. 10–9 Torr. The analysis results are expressed in atomic percentages. Two treated and cleaned samples were tested by XPS

2.3. Analysis of surface topography by SEM (scanning electron microscopy)

The surface topography of the implants was evaluated by scanning electron microscope. Analysis was conducted using an EVO MA 10 SEM (Zeiss). The electron acceleration voltage was maintained at 15 kV, the working distance at 15 mm. These parameters are reported in the images, along with the level of magnification (MAG) and the kind of detector utilized (Signal A = SE1 or CZ BSD).

Images were acquired in both conventional mode (Signal A = SE1) and in backscattered electron mode (Signal A = CZ BSD), allowing improved contrast between different chemical elements.

Stereo-SEM (SSEM), using dedicated software to convert conventional SEM images into three-dimensional data (Mex 4.2, Alicona Imaging), evaluated roughness quantitatively. In particular, this evaluation exploits the basic principle of stereovision. Basically, two images of the same field of view are acquired after eucentric rotation by a given angle. This is obtained by changing the angle between the sample and the electrons, by tilting the table that holds the sample. The tilting angle is set and controlled by the instrument control software. The couple of images obtained (stereopair), the size of the field of view and the tilting angle are the incoming data, that the software converts into a single three-dimensional image, where each data point is characterized by the values of the x, y, z coordinates. The image obtained by this process allows then to measure height profiles (roughness profiles) and to calculate the different roughness parameters defined by relevant literature and standards.

2.4. Dental pulp extraction and differentiation

Human dental pulps were extracted from healthy molar teeth, which had been extracted because of mucosal inflammation (impacted teeth with pericoronitis) or for orthodontic reasons from adult subjects aged 16 to 0.66. The pulps were classified into 6 age groups (12 teeth per group). Each subject gave informed written consent for the use of his or her donor of dental pulps. The Ethical Committee of Padua Hospital approved the research protocol. Before extraction, each subject was checked for systemic and oral infections or diseases. Only disease-free subjects were selected for pulp collection. Each subject was pretreated for 1 week with professional dental hygiene. Before extraction, the dental crown was covered with a 0.3% chlorexidine gel (Forhans, New York, NY) for 2 min. After mechanical fracturing, dental pulp was obtained by means of a dentinal excavator or a Gracey curette. The pulp was gently removed and immersed for 1 h at 37uC in a digestive solution: 100 U/mL penicillin, 100 mg/mL streptomycin, 0.6 mL of 500 mg/mL clarithromycin, 3 mg/mL type I collagenase, and 4 mg/mL dispase in 4 mL of 1 M PBS. Once digested, the solution was filtered through 70 mm Falcon strainers (Becton & Dickinson, Franklin Lakes, NJ) [15].

2.5. Immunocytochemical staining

DPSCs were layered over cover slip, fixed with absolute acetone for 10 min at room temperature and cryopreserved at −20C° until use. The following markers were visualized with immunofluorescence: SH2, SH3, SH4, CD14, CD34; CD45 (monoclonal mouse anti-human; SIGMA). Briefly, after non-specific antigen sites were saturated with 1/20 serum in 0.05 M maleate

TRIZMA (Sigma; pH 7.6) for 20 s, 1/100 primary monoclonal anti-human Ab (Sigma) was added to the samples. After incubation, immunofluorescence staining was performed with fluorescein (anti-mouse) secondary antibody.

2.6. Karyotype analysis

After 45 days of culture on DPSc were exposed to colchicine (Sigma-Aldrich, St. Louis, MO, USA) for 6 h, washed in PBS, dissociated with trypsin (Lonza S.r.l), and centrifuged at 300 g for 5 min. Colchicine is need to perform karyotyping analyses of the cells, because we need to stop cell mitosis in the metaphase stage. It is during this stage of nuclear division that the chromosomes are most condensed and, as a result, visible with a light microscope. After the cells have been arrested in this stage, they are then placed into a hypotonic solution, which causes water to enter and enlarge the cells. The cells are then placed into a chemical fixative to maintain this condition. Following this procedure, the cells can be “splatted” onto microscope slides, stained, and viewed microscopically.

The pellet was carefully suspended and incubated in 1% sodium citrate for 15 min at 37 °C, then fixed and spread onto −20 °C cold glass slides. Metaphases of cells were Q-banded and karyotyped in accordance with the international system for human cytogenetic nomenclature recommendations. Twenty-five metaphases were analyzed for three expansions.

2.7. Growth curve and doubling time

Cells were seeded into the implants at an initial density of 5×10^4. When cells reached confluence, they were detached, counted and re-seeded at a density of 5×10^4. The PDT of the cells was calculated according to the formula:

$$PDT = (T0-T1)\log 2/(\log N_t - \mathrm{Log}Nt_1)$$

where PDT represents the cell doubling time, t represents the duration