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Surface biocompatibility of differently textured titanium implants with mesenchymal stem cells

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ABSTRACT

Objective. The major challenge for contemporary dentistry is restoration of missing teeth; currently, dental implantation is the treatment of choice in this circumstance. In the present study, we assessed the interaction between implants and Dental Pulp Stem Cells (DPSCs) *in vitro* by means of 3D cell culture in order to better simulate physiological conditions.

Methods. Sorted CD34$^+$ DPSCs were seeded onto dental implants having either a rough surface (TriVent) or one coated with a ceramic layer mimicking native bone (TiUnite). We evaluated preservation of DPSC viability during osteogenic differentiation by an MTT assay and compared mineralized matrix deposition with SEM analysis and histological staining; temporal expression of osteogenic markers was evaluated by RT-PCR and ELISA.

Results. Both surfaces are equally biocompatible, preserve DPSC viability, stimulate osteogenic differentiation, and increase the production of VEGF. A slight difference was observed between the two surfaces concerning the speed of DPSC differentiation.

Significance. Our study of the two implant surfaces suggests that TriVent, with its roughness, is capable of promoting cell differentiation a bit earlier than the TiUnite surface, although the latter promotes greater cell proliferation.

1. Introduction

Edentulism can be considered the basis of chronic esthetic and functional deterioration of the oral cavity due to progressive resorption of the maxillary bony structures, resulting in ptosis of the overlying soft tissues. Importantly, tooth loss produces progressive deficiency in the normal functions of the mouth, such as chewing, swallowing, and phonation [1]. The main causes of tooth loss are traumatic events,

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endodontic pathologies, neglecting caries, and, especially, periodontal disease. Tooth loss represents a major challenge for contemporary dentistry, which recognizes dental implantation as the treatment of choice in this situation in order to restore masticatory function and the esthetics of the patients [2].

Implant design has a key role in osteointegration, a direct structural and functional connection between living bone and the surface of the implant [3]. An implant is integrated, and hence capable of supporting the load placed upon it, when all the gaps at the bone–implant interface are filled with newly synthesized bone tissue. This process is carried out by Mesenchymal Stem Cells (MSCs) [4]. MNCs have self-renewal properties and are capable of differentiating into many cellular lineages [5] upon appropriate induction [6]. Immediately after implant insertion, local blood vessel growth allows the recruitment of migratory MSCs to the surgical site and the implant's surface. The cells then proliferate and differentiate into mature osteoblasts responsible for bone matrix deposition, a process essential for perfect implant integration. Bone formation around the implant depends on the level of differentiation of the MSCs. This process is largely affected by the nature of the implant: in fact, studies have reported that chemical, mechanical, and topographic characteristics of the implant surface influence all events involved in bone formation at the bone–implant interface, including cell adhesion, proliferation, differentiation, and matrix deposition [7]. For these reasons, many implant textures – obtained through anodization, calcium phosphate coating, chemical or biological modification, sandblasting, *etc.* – have been designed and studied *in vitro* and *in vivo* over the last decades in an attempt to improve osteointegration [8–11].

Today, titanium and its alloys are recognized as the material of choice in implant dentistry because of their excellent biologic and biomechanical properties, their biocompatibility, and the ease with which different textures can be produced [12]. Roughness is the most-studied characteristic of implant texture, and it has been reported that this feature is important in enhancing osteoblast differentiation, with increased roughness maximizing osteoinduction [13]. However, reports of the effects of titanium surface roughness on cell differentiation are contrasting [14].

Therefore, we decided to investigate how implant texture affects the early phases of osteodifferentiation *in vitro*. The study was conducted with Dental Pulp Stem Cells (DPSCs), since they are easily extracted with high efficiency from dental pulp [15]; moreover, DPSCs are largely employed in tissue engineering because of their capability to differentiate into many cells types, such as mature osteoblasts [5,16], and due to their ability to generate a three-dimensional (3D) bone tissue *in vitro* [17]. Moreover, these cells have been recently investigated also with regards to their specific migratory capabilities in relationship with other stem cells [18].

In addition, these cells are naturally delegated to dental tissue repair: in fact, DPSCs have been successfully employed in bone repair in *vitro* [19,20] and *in vivo* [21,22].

2. Materials and methods

2.1. Dental implants

The dental implants used in this study were Tri-Vent, purchased from TRI Dental Implants Int AG (Baar, Switzerland), and TiUnite, purchased from Nobel Biocare (Nobel Biocare Goteborg, Sweden). Tri-Vent is a sandblasted, acid-etched surface created by blasting the implant surface under pressure; TiUnite is composed of a slightly rough titanium oxide layer covered with a phosphorus coat conferring it a ceramic-like property rich in micropores.

2.2. Cell extraction and 2D culture

Mesenchymal stem cells were obtained by the extraction of dental pulp tissue from third molars of 15 healthy patients (age range: 20–35 years old) as described previously [23]. All subjects signed the Ethical Committee consent brochure (Second University Internal Ethical Committee). Each subject underwent professional dental hygiene treatments for a week before tooth extraction. Only infection-free subjects were selected for cell collection. After mechanical and enzymatic digestion of the tissue with a collagenase I/dispase solution, the sample was filtered with 70 μm Falcon strainers (BD Pharmingen, Buccinasco, Milano, Italy) and centrifuged for 7 min at 1300 rpm. The pellets were then plated in T-25 flasks at 37 °C and 5% CO_2 in DMEM culture medium supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and 100 U/ml penicillin and 100 mg/ml streptomycin (all purchased from GIBCO-Life Technologies, Monza, Italy). Adhered cells were expanded until they reached about 5×10^5 cells/flask.

2.3. FACS analysis and sorting

Cells were detached using trypsin–EDTA (GIBCO). At least 200,000 cells were incubated with fluorescent-conjugated antibodies for 30 min at 4 °C, washed, and re-suspended in PBS. The antibodies used in this study were: anti-CD34 PE (BD Pharmingen, Buccinasco, Milano, Italy) and anti-CD90 FITC (BD Pharmingen, Buccinasco, Milano, Italy). Isotypes were used as controls. Cells were analyzed with an Accuri C6 (BD Biosciences, San Jose, CA, USA) and data collected with FSC Express version 3 (De Novo Software). Cells were sorted using simultaneous positivity for CD90 and CD34 using a FACS ARIA III (BD, Franklin Lakes, NJ, USA). The purity of sorted populations was routinely 90%.

2.4. 3D cell culture: In vitro tissue engineering

After proliferation, collected subpopulations were seeded – at a density of 5×10^5 cells/implant – onto dental implants that had been previously washed in PBS. After 1 h of incubation in 100 μl of culture medium to allow cell attachment, the cell–implant devices were transferred to 15 ml tubes with a cap filter and incubated with osteogenic medium in a humidified atmosphere at 37 °C and 5% CO_2 in a rotating culture apparatus (Wheaton Science Products, Millville, NJ, USA) at 6 rpm; cells plated in flasks were used as control. The 3D culture was