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Human gingival fibroblasts function is stimulated on machined hydrided titanium zirconium dental implants

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ABSTRACT

Objectives: The aim of this study was to evaluate the influence of different titanium zirconium (TiZr) alloy surfaces on primary human gingival fibroblasts (HGF) for improved soft tissue integration of dental implants.

Methods: TiZr polished, machined and machined + HCl/H_2SO_4 acid-etched surfaces were modified by cathodic polarization and/or HNO_3/HF acid etching. Contact angle of surfaces was measured. The influence of modified TiZr surfaces on HGF was evaluated through the analysis of cell number, morphology, recovery after a wound (wound healing assay) and the expression of several genes, including matrix metalloproteinase-1 (MMP1) and metallopeptidase inhibitor-1 (TIMP1).

Results: Modification of TiZr surfaces decreased its hydrophilicity. Hydride implementation on TiZr surfaces *via* cathodic polarization increased TIMP1 expression and decreased MMP1/TIMP1 mRNA ratio. Cathodic polarization of machined surfaces promoted cell attachment. Cells on machined and machined + cathodic polarization surfaces grew aligned to the microgrooves whereas on all polished surfaces they grew randomly. Acid etching of polished and machined surfaces did not improve HGF function.

Conclusions: Hydride implementation on TiZr machined surfaces may be used as new dental implant material for improved soft tissue integration.

Clinical significance: Enhancing dental implant surfaces' bioactivity by hydride implementation may promote soft tissue attachment and sealing around the implant and reduce peri-implantitis related to ECM-destruction compared with conventional machined surfaces.

1. Introduction

The integration of the dental implant intra-osseous component and the transmucosal component (abutment) with the hard tissue (bone) and the peri-implant mucosa (gingiva) is essential in order to minimize dental implant failure or complications. While during the past years research and development of dental implant biomaterials has been focused on osseointegration, nowadays soft tissue integration is one of the frontiers in dental implant research, as dental implants require a soft tissue barrier to prevent bacterial penetration[1]

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and also to inhibit epithelial downgrowth.[2] After installation of a dental implant, fibroblasts from the oral connective tissue (gingival fibroblasts) are the preferred cells to form a healthy and collagen-rich connective tissue to repopulate the wound and attach to the abutment of the implant.[3]

Titanium-zirconium (TiZr) alloys have shown increased corrosion resistance,[4] improved tensile and fatigue strength,[5–7] similar biocompatibility,[8–10] higher integrin-β3 expression[11] in comparison with titanium (Ti), the gold standard in implantology,[12] and have been suggested as potential clinical candidates to improve soft tissue integration. Moreover, Ti properties are limited in the case of small diameter implants when being placed in narrow bone space (*e.g.* maxilla front).[13]

Surface topography and chemistry affect fibroblasts attachment, proliferation and differentiation[1,14] and are critical variables in determining the soft tissue response to a biomaterial.[15] Surface topography of dental implants can be altered by different processes, producing micro and nano-scale topographic features. Fibroblasts prefer smooth surfaces to rough surfaces,[14,16,17] although finely grooved surfaces perform better than smooth ones.[17–21] In other studies, fibroblast proliferation increased on micro-grooved acid-etched surfaces compared with smooth surfaces.[22–24] *In vivo* studies indicate that a certain surface roughness is required for the formation of a stable soft tissue seal around the abutments[25] and that micro-textured surfaces perpendicularly oriented to the migration direction of epithelial tissue impede its downgrowth.[26]

Hydride implementation to Ti surfaces has been reported in earlier studies,[27] and shown to promote implant retention in bone[28] and gingival fibroblasts proliferation.[29] TiZr showed 1.9 times greater surface hydrogen concentration than Ti when etched in acid, suggesting that Zr enhance hydride formation on Ti alloys.[30] Moreover, TiZr showed homogeneously distributed nano-spheres at a size of 80–100 nm after hydride implementation.[31]

In previous studies, we produced different TiZr surfaces by polishing, machining, and also using cathodic polarization and acid etching,[32] resulting in different topographies (smooth, micro-grooved and rough) with surface area roughness (S_a) ranging from 29 to 214 nm. These modifications, although producing desired topographies, altered surface chemistry, showing increased hydride content, which could condition cell behaviour on TiZr. We hypothesized that hydride implementation on TiZr surfaces could influence positively on human gingival fibroblasts functions. The aim of this paper was to evaluate short and long term influence of modified TiZr surfaces on human gingival fibroblasts to select the best physical–chemical characteristics of TiZr for dental implant abutments that could reinforce soft tissue integration and, therefore, could have a clinical use.

2. Materials and methods

2.1. Samples preparation

TiZr coin-shaped samples (diameter 4.39 mm, thickness 2 mm) containing 13–17% Zr were provided by Institut Straumann (Basel, Switzerland) as machined (M) and machined + HCl/H_2SO_4 acid etched hydrophilic (ME_S) surfaces.[33] Then, surfaces were modified by polishing and/or cathodic polarization at 15 mA/cm^2 for 5 h (hydridation)[27,28] and further modified by HNO_3/HF acid etching (pickling), as previously described.[32] Eight groups were created for the study (Table 1).

Table 1 – Groups and surface modifications used in the study.

Group	Modification
P	Polished
PH	Polished hydrided
PE_FH	Polished, HNO_3/HF acid-etched and hydrided
M	Machined
MH	Machined hydrided
ME_FH	Machined, HNO_3/HF acid-etched and hydrided
ME_S	Machined and HCL/H_2SO_4 acid-etched
ME_SH	Machined, HCL/H_2SO_4 acid-etched and hydrided

2.2. Contact angle measurement

Contact angle of the different TiZr surfaces was measured as an index of hydrophilicity. Coin-shaped samples were washed under Milli-Q water stream and air-dried before contact angle measurement using Contact Angle System OCA (Dataphysics Instruments GmbH, Filderstadt, Germany) with sessile drop (needle in) mode at room temperature. Two coins from each group and two areas of each surface were measured ($n = 4$) with 0.5 μl ultrapure water.

2.3. Cell culture

Primary human gingival fibroblasts (27 years, Caucasian, female, lot number 313X100401, Provitro GmbH, Berlin, Germany) were used. HGF were cultured at 37 °C in a humidified atmosphere of 5% CO_2, and maintained in fibroblast growth medium (Provitro GmbH) supplemented with 10% foetal calf serum (FCS) and 50 ng amphotericin/ml and 50 μg gentamicin/ml (Provitro GmbH). Culture media was changed every other day. Cells were subcultured before reaching confluence using phosphate buffered saline (PBS) and Trypsin/EDTA (PAA Laboratories, Pasching Austria), as recommended by the supplier. Trypan blue stain was used to determine total and viable cell number. Experiments were performed with HGF cells between passage seven and eight from the initial isolation.

To test the performance of the different surfaces, coin-shaped implant abutments were dipped in PBS before placing them in 96-well half area plates (Corning, Lowell, MA, USA). The number of cells seeded on each TiZr sample and in empty wells of the same plate was3.5×10^3. HGF cells were maintained up to 14 days in complete fibroblast growth medium.

2.4. Cell number determination

The number of cells on each sample was determined by DNA quantification ($n = 4$). Culture media was removed from wells