"Micropatterning zirconia surfaces to improve contact with bone cells"

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Teeth lost due to factors such as caries and periodontitis remains as one of the most diffused health problems, as stated by the World Health Organization (WHO). Since the introduction of dental implants in the eighties that Titanium became the gold standard for restorative therapy, bearing in mind the low biologic cost in comparison with alternative methods such as fixed prosthesis of several units. Its major success is due to initial osteointegration and long-term survival rate.

In recent years a significant effort has been put in producing all ceramic dental implants, based in yttria stabilized zirconia (Y-TZP). Although some commercial products consisting of all ceramic implants have gained some portion of the world implant market, there are still many question marks regarding biological, mechanical and economical aspects. In the present work the following fundamental question was raised: How would it be possible to improve the surface response of all ceramic dental implants? This is a very broad question, leading to the chance to exploit different scenarios, including strategies for guided tissue regeneration in periodontics but also for other interesting applications such as craniofacial reconstruction.

Understanding the mechanisms of cell-surface interaction is essential for the design of biomaterials and successful tissue engineering strategies. It is well established that micron sized topography greatly affects cell-surface interactions. Different surface topographies can influence behaviours such as adhesion, morphology, orientation, migration and differentiation.
With this knowledge, the main goal of this work was to modify the surface of 3Y-TZP ceramics through micropatterned silica coatings, obtained using the synergy between sol-gel technology and microstamping. These surfaces were developed aiming at improving osteointegration and controlling the bacterial adhesion to the implant. To reach such aims, the present work concerns the production of zirconia substrates to apply bioactive and micropatterned SiO_2 coatings to control cell attachment, spreading, proliferation and differentiation.
Different isotropic and anisotropic micropatterned coatings were obtained by stamping silica on the surface of the zirconia, using PDMS negative molds, obtained from a soft-lithography method.
Flat silica coatings were prepared by spin- and dip-coating techniques to use as controls in all the characterization studies. The introduction of nanohydroxyapatite aggregates to the thin films, for higher bioactivity, was also evaluated. Both the zirconia substrate and

the silica coatings were characterized in terms of hydrophobicity, roughness, morphology and chemical composition. The thin films adhesion to the substrate was also evaluated using a scratch test.
The evaluation of *in vitro* biocompatibility of the developed materials was performed using several types of human cell cultures (MSCs, fibroblasts, osteoblasts, endothelial). The bacterial adhesion to the silica thin films was characterized using different bacterial strains from the oral cavity.
The results obtained point out to adequate silica based microtextured coatings produced by the combination of sol-gel/soft-lithography technologies.
Isotropic and anisotropic micropatterned silica thin films were successfully obtained. The anisotropic line-shaped and the isotropic pillars micropatterns faithfully reproduced the mold features with several interspacing's from 5 μm to 45 μm.

From the *in vitro* biological evaluation of the several types of eukaryotic cells in different substrate/coating combinations, it was possible to show that, in the initial phases of adhesion and proliferation, all cell types presented directional alignment with the anisotropic coatings, this effect being reduced when the inter-spacing was 45 μm. This alignment was less evident for isotropic coatings, where cells show a more spread morphology.
Besides, with some cell types, the effect of cell alignment control was not observed for culture times over 14 days of proliferation; however the available information allows to estimate that this effect is lost when cell multilayers are established, leading to a more natural organization such as the one in bone tissue formation.
The preliminary results of human dental pulp derived MSCs motility on micropatterned surfaces, obtained by time-lapse microscopy, have shown a higher motility and directionality in the movements of cells lying on patterned surfaces when compared to similar flat surfaces.
Osteogenic differentiation assays with human bone marrow stromal cells and human dental pulp derived MSCs have also shown that the micropatterned surfaces induced a higher osteogenic differentiation when compared with the flat silica controls, observed by the higher ALP activity, production of extracellular matrix and mineralization and expression of osteogenesis genes by RT-PCR.
The results on the bacterial adherence using quasi-static culture systems were not conclusive. The patterned surfaces presented a higher bacterial adherence on adhesion assays. However, there were no significant differences between the line-shaped and flat surfaces. Presumably this will require the use in the future of flux technologies with reactors or of microfluidics techniques. Also, biofilms formation studies are important to understand the influence of the micropatterns during bacteria proliferation.

After implantation, a competition exists between integration of the material into the surrounding tissue and bacterial adhesion to the implant surface. With micropatterned materials this difficulty may be overcome, taking into account the higher cell proliferation observed.
The synergy between micropatterning and sol-gel technology allows for the exploitation of new surface modification alternatives for dental implants.