Cellular alignment induction during early in vitro culture stages using micropatterned glass coatings produced by sol-gel process

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Keywords: Micropatterning, sol-gel, contact guidance

Abstract. Cell behaviour such as adhesion, morphology, proliferation and functional activity are highly influenced by surface properties including hydrophobicity, roughness, texture and morphology. These surface properties may be controlled using a mixture of additive coating techniques to produce glass coatings by sol-gel process and soft lithography on dental ceramics.

The purpose of this work was to compare cell adhesion and early orientation of Human Bone Marrow (HBM) cells cultured on micro-patterned (micro-PGC) and on flat glass coatings (FGC) produced by sol-gel processing.

Spin coating was used to apply SiO₂ flat coatings on glass substrates as model surfaces. Photolithography was applied to produce master patterns with microscale dimensions. A moulding technique was used to print micropatterned SiO₂ glass coatings produced by a sol-gel process. The coatings were then sintered, sterilized and cultured with HBM cells derived from primary cultures, using a standardized protocol, for 1 and 7 days. Cell morphology and orientation were observed using scanning electron microscopy (SEM) and confocal laser scanning microscopy (CLSM).

Flat and MPGC with line shaped features were produced. Cells presented a typical osteoblastic morphology on flat surfaces while slimmer, preferentially oriented and more elongated morphologies could be seen on line micro-patterned surfaces. HBM cells cultured on flat glass coatings showed increased tendency to spread and to assume more randomized proliferation when compared to the cells on the micro-patterned glass coatings. Micro-patterned glass coatings showed higher orientation control and smaller delay in the rate of proliferation, in early stages of in vitro culture as compared to flat coatings.

These preliminary studies revealed that Micro-PGC induce significant morphological changes and controlled orientation of HBM cells during early stages of cell proliferation.
Introduction.

Titanium is the material of choice for many dental implant applications. Some titanium implants do not integrate, have poor long term survival, and may be affected by peri-implantitis or have poor aesthetics [1, 2]. Recent developments in dental ceramics have introduced yttria stabilized zirconia as a ceramic material with high mechanical strength, improved aesthetics, [3] and minimal cytotoxic or mutagenic effects [4, 5]. However, this material presents reduced bioactivity. Furthermore, there is a lack of information regarding long-term clinical success rates for dental applications [6].

Peri-implantitis is generally defined as a chronic inflammation of the support tissues around the implant produced by bacterial colonization, facilitated by the implant/abutment gap, chemical composition and/or surface roughness of the restorative components [7].

Low bacterial adherence and osteointegration may be obtained with a microfabrication strategy such as soft lithography and bioactive glass based nanostructured coatings by sol-gel process onto ceramic components like the abutments and screws. Surface texture of dental implants affects the rate of osteointegration and biomechanical fixation [8].

During the first half of the last century it was discovered that cell behaviour was affected by the topographical morphology of the surfaces. This phenomenon was first named as stereotropism and later became known as contact guidance. Brunette was among the first to propose the use of micro-grooved implant surfaces to prevent apical epithelial migrations [9].

Glass coatings applied by sol-gel process [10] and soft lithography on ceramics have produce surfaces where control of chemistry, roughness, thickness and textures are possible [11-13]. In this work, the combination of microfabrication techniques and sol-gel processing was used to produce specifically controlled surfaces for topographical modulation studies on cell spreading on ceramic biomaterials. We compared early orientation of Human Bone Marrow (HBM) cells on micro-patterned (micro-PGC) and on flat glass coatings (FGC) produced by sol-gel processing were compared.

Materials and Methods

The sol-gel process was used to produce SiO₂ flat and micropatterned glass coatings using hybrid silica sols resulting from acid catalysis in a single stage. Tetraethylorthosilicate (TEOS, Aldrich) and methyltriethoxysilane (MTES, Aldrich) were selected as silica precursors for the sol, while alcohol was used as a solvent and nitric acid and acetic acid were used as catalysts. Spin coating technique was chosen to apply SiO₂ flat coatings on microscope glass slides as model substrates.

![Figure 1](image.png)

Figure 1. Micromoulding process: a) Preparation of substrate (1), liquid drop of SiO₂ solution prepared by Sol-Gel process (2) and PDMS micromoulding template (3) produced by soft lithography. b) Pressed with constant pressure and aged for 2 hours and c) demoulding and sintering.

Photolithography was used to produce master patterns with microscale dimensions. A polydimethylsiloxane (PDMS) moulding was obtained from the masters by uniformly mixing PDMS (Silastic T-2, Dow Corning, USA) with a curing agent, pouring the mixture onto the masters,
degassing, and curing [14]. Figure 1 shows the process for fabricating the microapaterned coatings. A drop of sol was applied to the substrate. The PDMS mould was then applied and constant pressure was applied for 2 hours to solidify the structure [15]. The mould was removed, and the coatings were sintered using a thermal cycle that included heating up to 450 °C at a rate of 20 °C/min, a plateau at 450 °C for 30 min and cooling in air using an elevator furnace, followed by autoclave sterilization.

Human Bone Marrow (HBM) was obtained from patients undergoing orthopaedic surgery procedures. Informed consent to use this biological material, that would be otherwise discarded, was obtained. Bone marrow was cultured in α-minimal essential medium (α-MEM) containing 10% fetal bovine serum, penicillin-streptomycin (100 IU/ml and 10 mg/ml, respectively), fungizone (2.5 μg/ml) and ascorbic acid (50 μg/ml). Primary cultures were incubated in a humidified atmosphere of 5% CO₂ in air at 37 °C, and the medium was changed twice a week. Primary cultures were maintained for 10-15 days until near confluence, when adherent cells were enzymatically released with 0.04% trypsin and 0.025% collagenase.

Coated samples with and without micro-patterns were cultured with HBM cells (3rd and 5th passage), under the same conditions of primary cultures for 1 and 7 days. Cell morphology and orientation were followed using SEM and Confocal Laser Scanning Microscopy (CLSM) according to the following sequence: Three cultured samples per group were washed with PBS, fixed using 1.5% glutaraldehyde, dehydrated in graded alcohol solutions, gold sputtered and observed by SEM. For CLSM, three samples per group were washed with PBS, fixed in 4% v/v formaldehyde (methanol-free; Polyscience) for 15 min, permeabilized with 0.1% v/v Triton X-100 for 5 min, and incubated in 10 mg/ml bovine serum albumin and 100 μg/ml RNase for 45 min at room temperature. F-actin filaments were stained with Alexafluor-conjugated phalloidin (Molecular Probes) for 20 min and nuclei were counterstained with 10 μg/ml propidium iodide (Sigma) for 10 min. Finally, samples were washed with PBS and mounted and observed on a BioRad MRC 600 microscope and postprocessed with Leica Confocal Software (Leica, Mannheim, Germany).

Results and Discussions
Flat and MPGC with line shaped features with 5 μm ridge and groove width and ~3 μm height were produced (Fig. 2). The EDS analyses showed SiO₂ on both types of surfaces Fig. 2b.

Bone marrow cells were able to adhere and spread on both surfaces. Attached cells presented a spread – polygonal shape with cytoplasmic extensions adapting to the flat surface while slimmer,
preferentially oriented and more elongated morphologies could be seen on line micro-patterned surfaces.

Figure 3. SEM images of a) Flat SiO₂ coating and b) Micro-patterned SiO₂ coating with HBM cells cultured for 24h. c) CLSM image of interface between micropatterned (on the left side of the image) and flat coatings with HBM cells cultured for 7 days.

HBM cells cultured on flat glass coatings showed increased tendency to spreading and to assume more random proliferation distributions when compared to the cells on the micro-patterned glass coatings. HBM cells on micro-patterned glass coatings showed alignment parallel to long axis of ridges and smaller delay in the rate of proliferation in 7th day of culture compared to flat coatings (Fig. 3). These results are in agreement with other previously reported works [9].

Conclusions
These preliminary studies revealed that Micro-PGC induce significant morphological changes and controlled orientation of HBM cells during early stages of cell proliferation. The combination of sol-gel processing and microfabrication may provide a new means to modify the surface of implant materials to controlled cellular interactions.

Acknowledgments
The authors acknowledge Alban Program /E05D050652CO grant, FCT/SFRH/BD/36220/2007 grant and project FCT/POCI/SAU-BMA/56061/2004.

References